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(54) Title: NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

(57) Abstract: Disclosed herein are novel human nucleic acid sequences which encode polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies which immunospecifically-bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involding any one of these novel human nucleic acids and proteins.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

FIELD OF THE INVENTION

The invention generally relates to novel GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6 and GPCR7 nucleic acids and polypeptides encoded therefrom. More specifically, the invention relates to nucleic acids encoding novel polypeptides, as well as vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and polypeptides.

SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of novel nucleic acid sequences encoding novel polypeptides. The disclosed GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6 and GPCR7 nucleic acids and polypeptides encoded therefrom, as well as derivatives, homologs, analogs and fragments thereof, will hereinafter be collectively designated as "GPCRX" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated GPCRX nucleic acid molecule encoding a GPCRX polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids disclosed in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21. In some embodiments, the GPCRX nucleic acid molecule will hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of a GPCRX nucleic acid sequence. The invention also includes an isolated nucleic acid that encodes a GPCRX polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide comprising the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a GPCRX nucleic acid (*e.g.*, SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21) or a complement of said oligonucleotide.

Also included in the invention are substantially purified GPCRX polypeptides (SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22). In certain embodiments, the GPCRX

polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human GPCRX polypeptide.

The invention also features antibodies that immunoselectively-binds to GPCRX polypeptides, or fragments, homologs, analogs or derivatives thereof.

In another aspect, the invention includes pharmaceutical compositions that include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a GPCRX nucleic acid, a GPCRX polypeptide, or an antibody specific for a GPCRX polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a GPCRX nucleic acid, under conditions allowing for expression of the GPCRX polypeptide encoded by the DNA. If desired, the GPCRX polypeptide can then be recovered.

In another aspect, the invention includes a method of detecting the presence of a GPCRX polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby identifying the GPCRX polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of a GPCRX.

Also included in the invention is a method of detecting the presence of a GPCRX nucleic acid molecule in a sample by contacting the sample with a GPCRX nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to a GPCRX nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of a GPCRX polypeptide by contacting a cell sample that includes the GPCRX polypeptide with a compound that binds to the GPCRX polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a Therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting

disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, or other disorders related to cell signal processing and metabolic pathway modulation. The Therapeutic can be, *e.g.*, a GPCRX nucleic acid, a GPCRX polypeptide, or a GPCRX-specific antibody, or biologically-active derivatives or fragments thereof.

The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders or other disorders related to cell signal processing and metabolic pathway modulation. The method includes contacting a test compound with a GPCRX polypeptide and determining if the test compound binds to said GPCRX polypeptide. Binding of the test compound to the GPCRX polypeptide indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders or other disorders related to cell signal processing and metabolic pathway modulation by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a GPCRX nucleic acid. Expression or activity of GPCRX polypeptide is then measured in the test animal, as is expression or activity of the protein in a control animal which recombinantly-expresses GPCRX polypeptide and is not at increased risk for the disorder or syndrome. Next, the expression of GPCRX polypeptide in both the test animal and the control animal is compared. A change in the activity of GPCRX polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

In yet another aspect, the invention includes a method for determining the presence of or predisposition to a disease associated with altered levels of a GPCRX polypeptide, a GPCRX nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes measuring the amount of the GPCRX polypeptide in a test sample from the subject and comparing the amount of the polypeptide in the test sample to the amount of the GPCRX polypeptide present in a control sample. An alteration in the level of the GPCRX polypeptide in the test sample as compared to the control sample indicates the presence of or predisposition to a disease in the subject. Preferably, the predisposition includes, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. Also, the expression levels of the new polypeptides of the invention can be used in a method to screen for various cancers as well as to determine the stage of cancers.

In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject a GPCRX polypeptide, a GPCRX nucleic acid, or a GPCRX-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

In yet another aspect, the invention can be used in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the

present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode novel polypeptides. The novel nucleic acids and their encoded polypeptides are referred to individually as GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, and GPCR7. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "GPCRX".

The novel GPCRX nucleic acids of the invention include the nucleic acids whose sequences are provided in Tables 1A, 2A, 2C, 3A, 4A, 4C, 5A, 5C, 5E, 6A, and 7A inclusive ("Tables 1A - 7A"), or a fragment, derivative, analog or homolog thereof. The novel GPCRX proteins of the invention include the protein fragments whose sequences are provided in Tables 1B, 1C, 2B, 2D, 3B, 4B, 4D, 5B, 5D, 5F, 6B, and 7B inclusive ("Tables 1B - 7B"). The individual GPCRX nucleic acids and proteins are described below. Within the scope of this invention is a method of using these nucleic acids and peptides in the treatment or prevention of a disorder related to cell signaling or metabolic pathway modulation.

GPCR1

Novel GPCR1 is a G-protein coupled receptor ("GPCR") protein related to the cysteinyl leukotriene receptor. GPCR1 maps to human chromosome 13. The GPCR1 nucleic acid of 1260 nucleotides is shown in Table 1A. The GPCR1 open reading frame ("ORF") begins at one of two alternative ATG initiation codons, shown in bold in Table 1A. In one embodiment, the GPCR1 ORF begins with an initiation codon at nucleotides 105-107, and the encoded polypeptide is alternatively referred to herein as GPCR1a or as AL137118A. In another embodiment, the GPCR1 ORF begins with an ATG initiation codon at nucleotides 120-122, and the encoded polypeptide is alternatively referred to herein as GPCR1b or as CG54236-02. In either embodiment, the GPCR1 ORF terminates at a TAA codon at nucleotides 1143-1145. As shown in Table 1A, putative untranslated regions 5' to the start codon and 3' to the stop codon are underlined, and the start and stop codons are in bold letters.

Table 1A. GPCR1 nucleotide sequence (SEQ ID NO:1).

TGCTCCCTGTTTCATTAAAACCTAGAGAGATGTAATCAGTAAGCAAGAAGGAAAAAGGGAAATTCACAAAGTAACTTTTTGTGT
CTGTTTCTTTTTAACCCAGCATGGAGAGAAAATTTATGTCCTTGCAACCATCCATCTCCGTATCAGAAATGGAACCAAATGGCA
CCTTCAGCAATAACAACAGCAGGAACTGCACAATTGAAAACTTCAAGAGAGAATTTTTCCCAATTGTATATCTGATAATATTTT
TCTGGGGAGTCTTGGGAAATGGGTTGTCCATATATGTTTTCCTGCAGCCTTATAAGAAGTCCACATCTGTGAACGTTTTCATGC
TAAATCTGGCCATTTCAGATCTCCTGTTCATAAGCACGCTTCCCTTCAGGGCTGACTATTATCTTAGAGGCTCCAATTGGATAT
TTGGAGACCTGGCCTGCAGGATTATGTCTTATTCCTTGTATGTCAACATGTACAGCAGTATTTATTTCCTGACCGTGCTGAGTG
TTGTGCGTTTCCTGGCAATGGTTCACCCCTTTCGGCTTCTGCATGTCACCAGCATCAGGAGTGCCTGGATCCTCTGTGGGATCA
TATGGATCCTTATCATGGCTTCCTCAATAATGCTCCTGGACAGTGGCTCTGAGCAGAACGGCAGTGTCACATCATGCTTAGAGC
TGAATCTCTATAAAATTGCTAAGCTGCAGACCATGAACTATATTGCCTTGGTGGTGGGCTGCCTGCTGCCATTTTTCACACTCA
GCATCTGTTATCTGCTGATCATTCGGGTTCTGTTAAAAGTGGAGGTCCCAGAATCGGGGCTGCGGGTTTCTCACAGGAAGGCAC
TGACCACCATCATCATCACCTTGATCATCTTCTTCTTGTGTTTCCTGCCCTATCACACACTGAGGACCGTCCACTTGACGACAT
GGAAAGTGGGTTTATGCAAAGACAGACTGCATAAAGCTTTGGTTATCACACTGGCCTTGGCAGCAGCCAATGCCTGCTTCAATC
CTCTGCTCTATTACTTTGCTGGGGAGAATTTTAAGGACAGACTAAAGTCTGCACTCAGAAAAGGCCATCCACAGAAGGCAAAGA
CAAAGTGTGTTTTCCCTGTTAGTGTGTGGTTGAGAAAGGAAACAAGAGTATAAGGAGCTCTTAGATGAGACCTGTTCTTGTATC
CTTGTGTCCATCTTCATTCACTCATAGTCTCCAAATGACTTTGTATTTACATCACTCCCAACAAATGTTGATTCTTAATATTTA

In one embodiment, the encoded GPCR1 protein is translated from nucleotides 105 through 1145 and has 346 amino acid residues, referred to as the GPCR1a protein. The GPCR1a protein was analyzed for signal peptide prediction and cellular localization. SignalP results predict that GPCR1a is cleaved between position 59 and 60 of SEQ ID NO:2, *i.e*, at the dash in the amino acid sequence GLS-IYV. Psort and Hydropathy profiles also predict that GPCR1 contains a signal peptide and is likely to be localized at the plasma membrane (certainty of 0.6000). The GPCR1a polypeptide sequence is presented in Table 1B using the one-letter amino acid code.

Table 1B. Encoded GPCR1a protein sequence (SEQ ID NO:2).

MERKFMSLQPSISVSEMEPNGTFSNNNSRNCTIENFKREFFPIVYLIIFFWGVLGNGLSIYVFLQPYKKSTSVNVFMLNLAISD
LLFISTLPFRADYYLRGSNWIFGDLACRIMSYSLYVNMYSSIYFLTVLSVVRFLAMVHPFRLLHVTSIRSAWILCGIIWILIMA
SSIMLLDSGSEQNGSVTSCLELNLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEVPESGLRVSHRKALTTIIIT
LIIFFLCFLPYHTLRTVHLTTWKVGLCKDRLHKALVITLALAAANACFNPLLYYFAGENFKDRLKSALRKGHPQKAKTKCVFPV
SVWLRKETRV

In an alternative embodiment, an encoded GPCR1 protein referred to alternatively as the GPCR1b or CG54236-02 polypeptide is translated from nucleotides 120 through 1145 and has a polypeptide sequence of 341 amino acid residues. The predicted GPCR1b polypeptide sequence includes amino acids 5 through 346 of SEQ ID NO:2 and is presented in Table 1C using the one-letter code. The identical predicted signal cleavage site in GPCR1a occurs in GPCR1b between position 54 and 55 of the sequence shown in Table 1C.

Table 1C. Encoded GPCR1b protein sequence.

MSLQPSISVSEMEPNGTFSNNNSRNCTIENFKREFFPIVYLIIFFWGVLGNGLSIYVFLQPYKKSTSVNVFMLNLAISDLLFIS
TLPFRADYYLRGSNWIFGDLACRIMSYSLYVNMYSSIYFLTVLSVVRFLAMVHPFRLLHVTSIRSAWILCGIIWILIMASSIML
LDSGSEQNGSVTSCLELNLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEVPESGLRVSHRKALTTIIITLIIFF
LCFLPYHTLRTVHLTTWKVGLCKDRLHKALVITLALAAANACFNPLLYYFAGENFKDRLKSALRKGHPQKAKTKCVFPVSVWLR
KETRV

Unless specifically addressed as GPCR1a or GPCR1b, any reference to a GPCR1 polypeptide or nucleic acid is assumed to encompass all variants.

GPCR1 was initially identified with a TblastN analysis of a proprietary sequence file for a G-protein coupled receptor probe or homolog which was run against the Genomic Daily Files made available by GenBank. A proprietary software program (GenScan™) was used to further predict the nucleic acid sequence and the selection of exons. The resulting sequences were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein.

In an analysis of sequence databases, it was found, for example, that the GPCR1 nucleic acid sequence has 269 of 422 bases (63%) identical to a *Gallus gallus* activated T cell-specific G protein-coupled receptor mRNA (GenBank Acc. No. L06109) (SEQ ID NO:23) shown in Table 1D. In all BLAST alignments herein, the "E-value" or "Expect" value is a numeric indication of the probability that the aligned sequences could have achieved their similarity to the BLAST query sequence by chance alone, within the database that was searched. For example, as shown in Table 1E, the probability that the subject ("Sbjct") retrieved from the GPCR1 BLAST analysis, in this case the *Gallus gallus* activated T cell-specific G protein-coupled receptor mRNA, matched the Query GPCR1 sequence purely by chance is 1.3×10^{-13}.

Table 1D. BLASTN of GPCR1 against activated T cell-specific GPCR

```
GENBANK-ID:CHKGPCR|acc:L06109 Gallus gallus activated T cell-specific G protein-
coupled receptor mRNA  (SEQ ID NO:23); Length = 1438
      Score = 509 (76.4 bits), Expect = 1.3e-13
      Identities = 269/422 (63%); Strand = Plus / Plus

Query:   186 AGCAGGAACTGCACAATTGAAAACTTCAAGA-G-AGAATTTTTCCCAAT-TGTATATCTG 242
             |||   |||||| | | |||  ||| |   | | | | ||| |     | ||| | || |
Sbjct:    91 AGCTCTAACTGCTCCACTGAGGACTCCTTTAAGTACACTTTGTATGGCTGTGTCT-TCAG 149

Query:   243 -ATAATATTTTTCTGGGGAGTCTTGGGAAA-TGGGTTGTCCATATATGTTTTC-CTGCAG 299
              ||  ||||| ||   ||  || | |  || || |||| | || ||  ||||  ||
Sbjct:   150 CATGGTATTTGTCCTCGGCCTCATAGCCAACTGCGTTG-CTATCTACATTTTTACTTTTA 208

Query:   300 CCTTATAAGAAGTCCACATCTGTGA--ACGTTTT-CATGCTAAATCTGGCCATTTCAGAT 356
             | ||  ||  ||| |  | | | ||  |||   | |||||| ||| |||| || || ||
Sbjct:   209 CATTG-AA--AGTGCGGAAC-GAGACCACGACGTACATGCTGAATTTGGCGATATCGGAC 264

Query:   357 CTCCTGTTCATAAGCACGCTTCCCTTCAGGGCTGACTATTATCTTAGAGGCTCC-AATTG 415
             || |||||  |    ||| | |||||||||  |  |||||| ||| | ||     || ||
Sbjct:   265 CTGCTGTTTGTGTTTACGTTGCCCTTCAGGA-T--CTATTA-CTTCGTGGTGAGGAACTG 320

Query:   416 GATATTTGGAGACCTGGCCTGCAGGAT-TATGTCTTATTCCTTGTATGTCAACATGTACA 474
             |   || |||||| |    |||| ||| |  |||    |  || ||   ||||||||||
Sbjct:   321 GCCCTTCGGAGACGTTCTGTGCAAGATCTCCGTCACGCTG-TTCTACACCAACATGTACG 379

Query:   475 GCAGTATT-TATTTCCTGACCGTGC-TGAGTGTTGTGCGTTTCCTGGCAATGGTTCACCC 532
             | || ||| |||| |||||||| ||| | || || |  || ||||||||  || || |||||
Sbjct:   380 GGAGCATTCTATT-CCTGACC-TGCATCAGCGTGGATCGCTTCCTGGCCATAGTGCACCC 437

Qu ry:   533 CTTTCGGCT-TCTGCATGTCACCAGCATCAGGAGTGCCTGGATCCTCTGTGGGATCATAT 591
             |||||| || |  |  | |   || ||  ||||  ||| ||||| | || | |    | |
Sbjct:   438 CTTTCG-CTCTAAGACTCTTCGCACCAAAAGGAACGCCAGGATCGTGTGCGTGGCGGTGT 496
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Query:   592 GGATCCTTATCATGGC 607
             |||||     |  ||||
Sbjct:   497 GGATCACCGTGCTGGC 512
```

In addition, the GPCR1 nucleic acid sequence has a 100% homology across 1260 nucleotides to the *Homo sapiens* cysteinyl leukotriene CysLT2 receptor (SEQ ID NO:24), as shown in Table 1E. The GenBank XM_007164 sequence (SEQ ID NO:24) was directly deposited to National Center for Biotechnology Information, NIH, Bethesda, MD 20894, USA, and provided to the public on November 16, 2000.

Table 1E. BLASTN of GPCR1 against CysLT2 receptor

ref|XM_007164 *Homo sapiens* cysteinyl leukotriene CysLT2 receptor; cDNA: PSEC0146 from clone PLACE1006979 (LOC57105), mRNA (SEQ ID NO:24)
Score = 2498 bits (1260), Expect = 0.0
Identities = 1260/1260 (100%); Strand = Plus / Plus

```
Query: 1    tgctccctgtttcattaaaacctagagagatgtaatcagtaagcaagaaggaaaaaggga 60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 160  tgctccctgtttcattaaaacctagagagatgtaatcagtaagcaagaaggaaaaaggga 219

Query: 61   aattcacaaagtaacttttgtgtctgtttctttttaacccagcatggagagaaaattta 120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 220  aattcacaaagtaacttttgtgtctgtttctttttaacccagcatggagagaaaattta 279

Query: 121  tgtccttgcaaccatccatctccgtatcagaaatggaaccaaatggcaccttcagcaata 180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 280  tgtccttgcaaccatccatctccgtatcagaaatggaaccaaatggcaccttcagcaata 339

Query: 181  acaacagcaggaactgcacaattgaaaacttcaagagagaatttttcccaattgtatatc 240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 340  acaacagcaggaactgcacaattgaaaacttcaagagagaatttttcccaattgtatatc 399

Query: 241  tgataatatttttctggggagtcttgggaaatgggttgtccatatatgttttcctgcagc 300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 400  tgataatatttttctggggagtcttgggaaatgggttgtccatatatgttttcctgcagc 459

Query: 301  cttataagaagtccacatctgtgaacgttttcatgctaaatctggccatttcagatctcc 360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 460  cttataagaagtccacatctgtgaacgttttcatgctaaatctggccatttcagatctcc 519

Query: 361  tgttcataagcacgcttcccttcagggctgactattatcttagaggctccaattggatat 420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 520  tgttcataagcacgcttcccttcagggctgactattatcttagaggctccaattggatat 579

Query: 421  ttggagacctggcctgcaggattatgtcttattccttgtatgtcaacatgtacagcagta 480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 580  ttggagacctggcctgcaggattatgtcttattccttgtatgtcaacatgtacagcagta 639

Query: 481  tttatttcctgaccgtgctgagtgttgtgcgtttcctggcaatggttcacccctttcggc 540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 640  tttatttcctgaccgtgctgagtgttgtgcgtttcctggcaatggttcacccctttcggc 699

Query: 541  ttctgcatgtcaccagcatcaggagtgcctggatcctctgtgggatcatatggatcctta 600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 700  ttctgcatgtcaccagcatcaggagtgcctggatcctctgtgggatcatatggatcctta 759

Query: 601  tcatggcttcctcaataatgctcctggacagtggctctgagcagaacggcagtgtcacat 660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 760  tcatggcttcctcaataatgctcctggacagtggctctgagcagaacggcagtgtcacat 819

Query: 661  catgcttagagctgaatctctataaaattgctaagctgcagaccatgaactatattgcct 720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct: 820  catgcttagagctgaatctctataaaattgctaagctgcagaccatgaactatattgcct 879

Query: 721  tggtggtgggctgcctgctgccatttttcacactcagcatctgttatctgctgatcattc 780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 880  tggtggtgggctgcctgctgccatttttcacactcagcatctgttatctgctgatcattc 939

Query: 781  gggttctgttaaaagtggaggtcccagaatcggggctgcgggtttctcacaggaaggcac 840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 940  gggttctgttaaaagtggaggtcccagaatcggggctgcgggtttctcacaggaaggcac 999

Query: 841  tgaccaccatcatcatcaccttgatcatcttcttcttgtgtttcctgccctatcacacac 900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1000 tgaccaccatcatcatcaccttgatcatcttcttcttgtgtttcctgccctatcacacac 1059

Query: 901  tgaggaccgtccacttgacgacatggaaagtgggtttatgcaaagacagactgcataaag 960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1060 tgaggaccgtccacttgacgacatggaaagtgggtttatgcaaagacagactgcataaag 1119

Query: 961  ctttggttatcacactggccttggcagcagccaatgcctgcttcaatcctctgctctatt 1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1120 ctttggttatcacactggccttggcagcagccaatgcctgcttcaatcctctgctctatt 1179

Query: 1021 actttgctggggagaatttaaggacagactaaagtctgcactcagaaaaggccatccac 1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1180 actttgctggggagaatttaaggacagactaaagtctgcactcagaaaaggccatccac 1239

Query: 1081 agaaggcaaagacaaagtgtgttttccctgttagtgtgtggttgagaaaggaaacaagag 1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1240 agaaggcaaagacaaagtgtgttttccctgttagtgtgtggttgagaaaggaaacaagag 1299

Query: 1141 tataaggagctcttagatgagacctgttcttgtatccttgtgtccatcttcattcactca 1200
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1300 tataaggagctcttagatgagacctgttcttgtatccttgtgtccatcttcattcactca 1359

Query: 1201 tagtctccaaatgactttgtatttacatcactcccaacaaatgttgattcttaatattta 1260
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1360 tagtctccaaatgactttgtatttacatcactcccaacaaatgttgattcttaatattta 1419
```

A BLASTX search was performed against public protein databases. As shown in Table 1F, the GPCR1a protein has 113 of 313 amino acid residues (36 %) identical to, and 177 of 313 residues (56 %) positive with, the 367 amino acid residue P2Y-like G-protein coupled receptor from *Homo sapiens* (ptnr:TREMBLNEW-CAA73144) (SEQ ID NO:25).

Table 1F. BLASTX of GPCR1a against P2Y-Like GPCR

```
>ptnr:TREMBLNEW-ACC:CAA73144 P2Y-Like G-Protein Coupled Receptor-Homo sapiens
(Human), 367 aa (SEQ ID NO:25)
Score = 477 (167.9 bits), Expect = 1.3e-44, P = 1.3e-44
Identities = 113/313 (36%), Positives = 177/313 (56%), Frame = +3.

Query:   135 SISVSEMEPNG---TFSNNNSRNCTIEN-FKREFFPIVYLIIFFWGVLGNGLSIYVFLQP 302
             |++  |+ | |    ||   +  |  |   +   |   ||+ |   ++|| |++++|++
Sbjct:    28 SMNGLEVAPPGLITNFSLATAEQCGQETPLENMLFASFYLLDFILALVGNTLALWLFIRD 87

Query:   303 YKKSTSVNVFMLNLAISDLLFISTLPFRADYYLRGSNWIFGDLACRIMSYSLYVNMYSSI 482
             +|  |  |||+++||++||  +  || |  |+  |++| ||++|||+  +  |+|||+||
Sbjct:    88 HKSGTPANVFLMHLAVADLSCVLVLPTRLVYHFSGNHWPFGEIACRLTGFLFYLNMYASI 147

Query:   483 YFLTVLSVVRFLAMVHPFRLLHVTSIRSAWILCGIIWILI-MASSIMLLDSGSSEQNGSVT 659
             ||||  +|  ||||+|||  +  | +     | + |  +|+++ +| + +|+    + |
Sbjct:   148 YFLTCISADRFLAIVHPVKSLKLRRPLYAHLACAFLWVVVAVAMAPLLVSPQTVQTNHTV 207
```

```
Query:   660 SCLELNLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEVPESGLRVSHR-- 833
             ||+|  |+  |      ++| |    || |   ||||||| |         ||||  |
Sbjct:   208 VCLQL--YR-EKASHHALVSLAVAFTFPFITTVTCYLLIIRSL------RQGLRVEKRLK 258

Query:   834 -KALTTIIITLIIFFLCFLPYHTLRTVHLTTWKV-GL-CKDRLHKALV--ITLALAAANA 998
             ||+  | | | || +||+|||  |+|++  ++  |  |  +   ||   ||  | + |
Sbjct:   259 TKAVRMIAIVLAIFLVCFVPYHVNRSVYVLHYRSHGASCATQRILALANRITSCLTSLNG 318

Query:   999 CFNPLLYYFAGENFKDRLKSAL 1064
               +|++|+|  | |+  | + |
Sbjct:   319 ALDPIMYFFVAEKFRHALCNLL 340
```

As shown in Table 1G, the GPCR1a protein was also found to have 346 of 346 amino acid residues (100%) identical to, and 346 of 346 residues (100%) positive with, the 346 amino acid sequence of *Homo sapiens* cysteinyl leukotriene CysLT2 receptor (ptnr:XP_007164) (SEQ ID NO:26). The cysteinyl leukotriene CysLT2 receptor (SEQ ID NO:26) is the protein encoded by GenBank XM_007164 sequence (SEQ ID NO:24), above, and was also directly deposited to National Center for Biotechnology Information, NIH, and made public on November 16, 2000.

Table 1G. BLASTX of GPCR1a against CysLT2 receptor

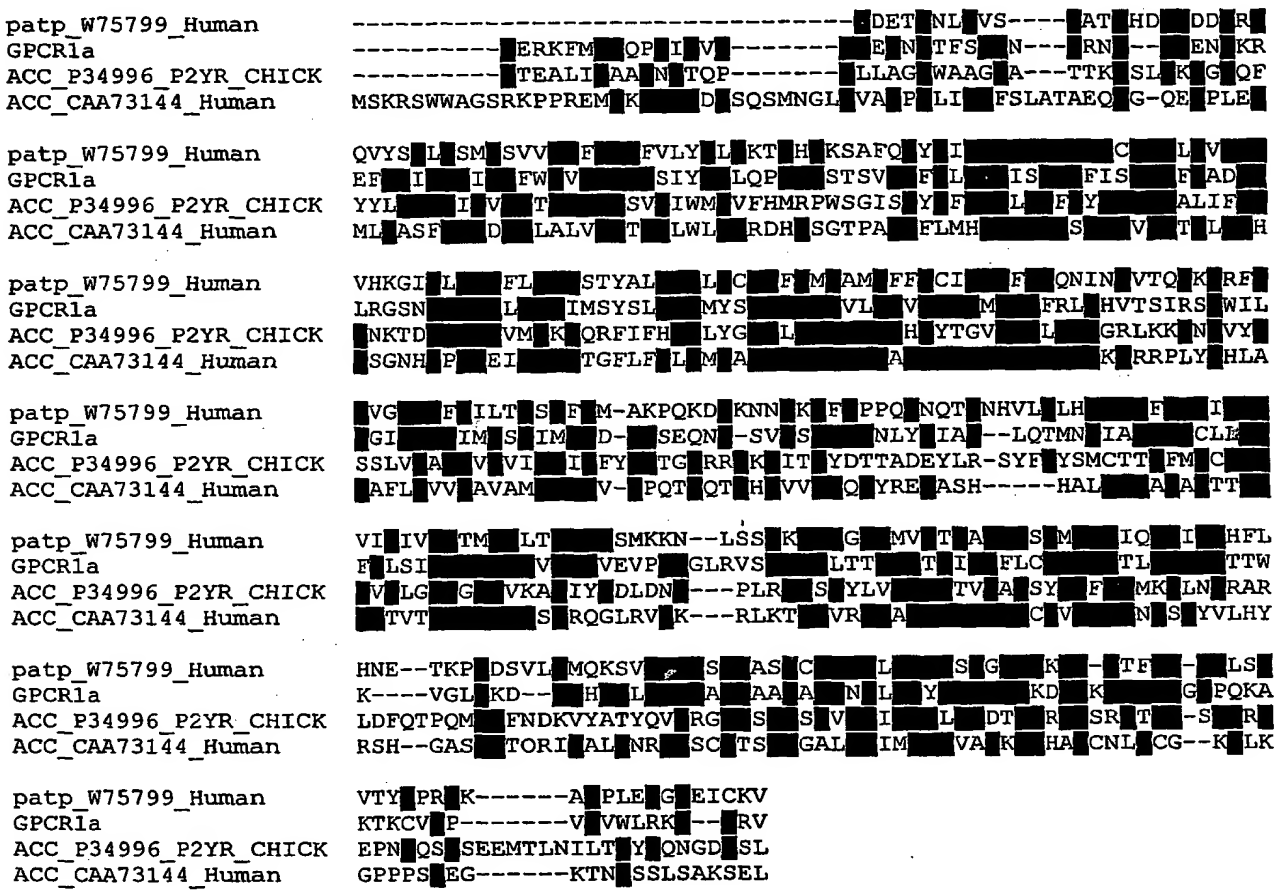
```
ptnr:XP_007164 cysteinyl leukotriene CysLT2 receptor; cDNA: PSEC0146 from
clone PLACE1006979 [Homo sapiens] (SEQ ID NO:26); Length = 346
 Score =  657 bits (1696), Expect = 0.0
 Identities = 346/346 (100%), Positives = 346/346 (100%)

Query: 1   MERKFMSLQPSISVSEMEPNGTFSNNNSRNCTIENFKREFFPIVYLIIFFWGVLGNGLSI 60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1   MERKFMSLQPSISVSEMEPNGTFSNNNSRNCTIENFKREFFPIVYLIIFFWGVLGNGLSI 60

Query: 61  YVFLQPYKKSTSVNVFMLNLAISDLLFISTLPFRADYYLRGSNWIFGDLACRIMSYSLYV 120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 61  YVFLQPYKKSTSVNVFMLNLAISDLLFISTLPFRADYYLRGSNWIFGDLACRIMSYSLYV 120

Query: 121 NMYSSIYFLTVLSVVRFLAMVHPFRLLHVTSIRSAWILCGIIWILIMASSIMLLDSGSEQ 180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 121 NMYSSIYFLTVLSVVRFLAMVHPFRLLHVTSIRSAWILCGIIWILIMASSIMLLDSGSEQ 180

Query: 181 NGSVTSCLELNLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEVPESGLRV 240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181 NGSVTSCLELNLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEVPESGLRV 240

Query: 241 SHRKALTTIIITLIIFFLCFLPYHTLRTVHLTTWKVGLCKDRLHKALVITLALAAANACF 300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 241 SHRKALTTIIITLIIFFLCFLPYHTLRTVHLTTWKVGLCKDRLHKALVITLALAAANACF 300

Query: 301 NPLLYYFAGENFKDRLKSALRKGHPQKAKTKCVFPVSVWLRKETRV 346
            |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 301 NPLLYYFAGENFKDRLKSALRKGHPQKAKTKCVFPVSVWLRKETRV 346
```

A ClustalW analysis comparing the protein of the invention with related protein sequences is given in Table 1H, with GPCR1 shown on line 2. In the ClustalW alignment of the GPCR1 protein, as well as all other ClustalW analyses herein, the black outlined amino acid residues indicate regions of conserved sequence (*i.e.*, regions that may be required to preserve structural or functional properties), whereas non-highlighted amino acid residues are

less conserved and can potentially be mutated to a much broader extent without altering protein structure or function.

Table 1H. ClustalW Analysis of GPCR1

1) patp_W75799_Human, SEQ ID NO:27
2) (AL137118A) Novel GPCR1a, SEQ ID NO:2
3) (sptr-ACC:P34996) P2Y Purinoceptor 1 (ATP Receptor) (P2Y1) (Purinergic Receptor) *Gallus gallus* (Chicken), 362 aa, SEQ ID NO:28
4) (STREMBL-ACC:CAA73144) P2Y-like G-Protein Coupled Receptor *Homo sapiens* (Human), 367 aa., SEQ ID NO:25



The presence of identifiable domains in GPCR1, as well as all other GPCRX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results, *e.g.*, for GPCR1 as disclosed in Table 1I, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. For Table 1I and all successive DOMAIN sequence alignments, fully conserved single residues are indicated by (*) and "strong" semi-conserved residues are indicated by (:). The "strong" group

of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 1I lists the statistics and domain description from DOMAIN analysis results against GPCR1. The region from amino acid residue 63 through 247 (numbered with respect to SEQ ID NO:2) most probably ($E = 3x10^{-30}$) contains a "seven transmembrane receptor (rhodopsin family) fragment" domain, aligned here with residues 1-177 of the 7tm_1 entry (SEQ ID NO:29) of the Pfam database. This indicates that the GPCR1 sequence has properties similar to those of other proteins known to contain this domain as well as to the 7tm_1 domain itself.

Table 1I. DOMAIN results for GPCR1

```
Sbjct: 7 transmembrane receptor (rhodopsin family) fragment (SEQ ID NO:29)
gnl|Pfam|pfam00001;  Length = 377
Score =  125 bits (315), Expect = 3e-30

Query:  63   GNGLSIYVFLQPYKKSTSVNVFMLNLAISDLLFISTLPFRADYYLRGSNWIFGDLACRIM  122
Sbjct:  1    GNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF  60
             ** *      :     *: *  :::**::***  : :     *      * *  : * *

Query:  123  SYSLYVNMYSSIYFLTVLSVVRFLAMVHPFR-LLHVTSIRSAWILCGIIWILIMASSIML  181
Sbjct:  61   VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVWVLSFTISCPM  120
                  :   :**  *  :*: *: *:  *       :* *   ::  *:*:*    *  :

Query:  182  LDSGSEQNGSVTSCLELNLYKIAKLQTMNYIALVVGCLLPFFTLSICYLLIIRVLLKVEV  241
Sbjct:  121  LFGLNNTDQN--ECIIA-------NPAFVVYSSIVSFYVPFIVTLLVYIKIYIVLRRRRK  171
             *   :  : :   *:                 : :*   :**    : *: *  ** :

Query:  242  PESGLR  247
Sbjct:  172  RVNTKR  177
               :  *
```

Expression information for GPCRX RNA was derived using tissue sources including, but not limited to, proprietary database sources, public EST sources, literature sources, and/or RACE sources, as described in the Examples. GPCR1 is expressed in at least the following tissues: adrenal gland/suprarenal gland, heart, placenta, spleen, and peripheral blood leukocytes.

The nucleic acids and proteins of GPCR1 are useful in potential therapeutic applications implicated in various GPCR- or OR-related pathologies and/or disorders. For example, a cDNA encoding the G-protein coupled receptor-like protein may be useful in gene therapy, and the G-protein coupled receptor-like protein may be useful when administered to a subject in need thereof. The novel nucleic acid encoding GPCR1 protein, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. The GPCRX nucleic acids and proteins are

useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, bronchial asthma, and other diseases, disorders and conditions of the like. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Additional GPCR-related diseases and disorders are mentioned throughout the Specification.

Further, the protein similarity information, expression pattern, and map location for GPCR1 suggests that GPCR1 may have important structural and/or physiological functions characteristic of the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration *in vitro* and *in vivo* (vi) biological defense weapon.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR1 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated GPCR1 epitope is from aa 30 to 60. In another embodiment, a GPCR1 epitope is from aa 80 to 95. In additional embodiments, GPCR1 epitopes are from aa 110 to 170, from aa 180 to 240; from aa 250 to 270, and from aa 280 to 305.

GPCR2

A second GPCR-like protein of the invention, referred to herein as GPCR2, is an Olfactory Receptor ("OR")-like protein. Two alternative novel GPCR2 nucleic acids and encoded polypeptides are disclosed.

In one embodiment, a GPCR2a variant (alternatively referred to herein as AC022289_A) includes the 1039 nucleotide sequence (SEQ ID NO:3) shown in Table 2A. A GPCR2a ORF begins with an ATG initiation codon at nucleotides 54-56 and ends with a TGA codon at nucleotides 996-998. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 2A, and the start and stop codons are in bold letters.

Table 2A. GPCR2a Nucleotide Sequence (SEQ ID NO:3).

ATATTTTGCTTTGGCAGGAACAATTCTCTTCAACCCTTCCATTAAAAGGAATT**ATG**ATGATGGTTTTAAGGAATCTGAGCATGG
AGCCCACCTTTGCCCTTTTAGGTTTCACAGATTACCCAAAGCTTCAGATTCCTCTCTTCCTTGTGTTTCTGCTCATGTATGTTA
TCACAGTGGTAGGAAACCTTGGGATGATCATAATAATCAAGATTAACCCCAAATTTCACACTCCTATGTACTTTTTCCTTAGTC
ACCTCTCTTTTGTTGATTTTTGTTACTCTTCCATTGTCACTCCCAAGCTGCTTGAGAACTTGGTAATGGCAGATAAAAGCATCT
TCTACTTTAGCTGCATGATGCAGTACTTCCTGTCCTGCACTGCTGTGGTGACAGAGTCTTTCTTGCTGGCAGTGATGGCCTATG
ACCGCTTTGTGGCCATCTGCAATCCTCTGCTTTATACAGTGGCCATGTCACAGAGGCTCTGTGCCCTGCTGGTGGCTGGGTCAT
ATCTCTGGGGCATGTTTGGCCCCTTGGTACTCCTTTGTTATGCTCTCCGGTTAAACTTCTCTGGACCTAATGTAATCAACCACT
TCTTTTGTGAGTATACTGCTCTCATCTCTGTGTCTGGCTCTGATATACTCATCCCCCACCTGCTGCTTTTCAGCTTCGCCACCT
TCAATGAGATGTGTACACTACTGATCATCCTCACTTCCTATGTTTTCATTTTTGTGACTGTACTAAAAATCCGTTCTGTTAGTG
GGCGCCACAAAGCCTTCTCCACCTGGGCCTCCCACCTGACTGCTATCACCATCTTCCATGGGACCATCCTTTTCCTTTACTGTG
TACCCAACTCCAAAAACTCTCGGCAAACAGTCAAAGTGGCCTCTGTATTTTACACAGTTGTCAACCCCATGCTGAACCCTCCGA
TCTACAGCCTAAGGAATAAAGACGTGAAGGATGCTTTCTGGAAGTTAATACATACACAAGTTCCATTTCAC**TGA**ACCAGTCTCA
AAAGTTGTTTTCAATCCAAATGAACAACCCA

The GPCR2a polypeptide (SEQ ID NO:4) encoded by SEQ ID NO:3 is 314 aa and is presented using the one-letter amino acid code in Table 2B. The Psort profile for GPCR2 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a GPCR2a peptide is between amino acids 43 and 44, *i.e.*, at the dash in the amino acid sequence VVG-NLG, based on the SignalP result.

Table 2B. GPCR2a protein sequence (SEQ ID NO:4)

MMMVLRNLSMEPTFALLGFTDYPKLQIPLFLVFLLMYVITVVGNLGMIIIIKINPKFHTPMYFFLSHLSFVDFCYSSIVTPKLL
ENLVMADKSIFYFSCMMQYFLSCTAVVTESFLLAVMAYDRFVAICNPLLYTVAMSQRLCALLVAGSYLWGMFGPLVLLCYALRL
NFSGPNVINHFFCEYTALISVSGSDILIPHLLLFSFATFNEMCTLLIILTSYVFIFVTVLKIRSVSGRHKAFSTWASHLTAITI
FHGTILFLYCVPNSKNSRQTVKVASVFYTVVNPMLNPPIYSLRNKDVKDAFWKLIHTQVPFH

The predicted GPCR2a sequence, above, was subjected an the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly

selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then used as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy, as described in the Examples.

The cloned sequence is disclosed as an alternative embodiment of GPCR2 (SEQ ID NO:5), referred to herein as the GPCR2b and reported in Tables 2C and 2D. GPCR2b is alternatively referred to herein as AC022289_A1. The GPCR2b ORF begins with an ATG initiation codon at nucleotides 54-56 and ends with a TGA codon at nucleotides 996-998. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 2C, and the start and stop codons are in bold letters.

Table 2C. GPCR2b Nucleotide Sequence

ATATTTTGCTTTGGCAGGAACAATTCTCTTCAACCCTTCCATTAAAAGGAATT**ATG**ATGATGGTTTTAAGGAATCTGAGCATGG
AGCCCACCTTTGCCCTTTTAGGTTTCACAGATTACCCAAAGCTTCAGATTCCTCTCTTCCTTGTGTTTCTGCTCATGTATGTTA
TCACAGTGGTAGGAAACCTTGGGATGATCATAATAATCAAGATTAACCCCAAATTTCACACTCCTATGTACTTTTTCCTTAGTC
ACCTCTCTTTTGTTGATTTTTGTTACTCTTCCATTGTCACTCCCAAGCTGCTTGAGAACTTGGTAATGGCAGATAAAAGCATCT
TCTACTTTAGCTGCATGATGCAGTACTTCCTGTCCTGCACTGCTGTGGTGACAGAGTCTTTCTTGCTGGCAGTGATGGCCTATG
ACCGCTTTGTGGCCATCTGCAATCCTCTGCTTTATACAGTGGCCATGTCACAGAGGCTCTGTGCCCTGCTGGTGGCTGGGTCAT
ATCTCTGGGGCATGTTTGGCCCCTTGGTACTCCTTTGTTATGCTCTCCGGTTAAACTTCTCTGGACCTAATGTAATCAACCACT
TCTTTTGTGAGTATACTGCTCTCATCTCTGTGTCTGGCTCTGATATACTCATCCCCCACTTGCTGCTTTTCAGCTTCGCCACCT
TCAATGAGATGTGTACACTACTGATCATCCTCACTTCCTATGTTTTCATTTTTGTGACTGTACTAAAAATCCGTTCTGTTAGTG
GGCGCCACAAAGCCTTCTCCACCTGGGCCTCCCACCTGACTGCTATCACCATCTTCCATGGGACCATCCTTTTCCTTTACTGTG
TACCCAACTCCAAAAACTCTCGGCAAACAGTCAAAGTGGCCTCTGTATTTTACACAGTTGTCAACCCCATGCTGAACCCTCTGA
TCTACAGCCTAAGGAATAAAGACGTGAAGGATGCTTTCTGGAAGTTAATACATACACAAGTTCCATTTCAC**TGA**ACCAGTCTCA
AAAG

The GPCR2b protein (SEQ ID NO:6) encoded by SEQ ID NO:5 is 314 amino acid in length, has a molecular weight of 35806.5 Daltons, and is presented using the one-letter code in Table 2D. As with GPCR2a, the most likely cleavage site for a GPCR2b peptide is between amino acids 43 and 44, *i.e.*, at the dash in the amino acid sequence VVG-NLG, based on the SignalP result.

Table 2D. GPCR2b protein sequence

MMMVLRNLSMEPTFALLGFTDYPKLQIPLFLVFLLMYVITVVGNLGMIIIIKINPKFHTPMYFFLSHLSFVDFCYSSIVTPKLL
ENLVMADKSIFYFSCMMQYFLSCTAVVTESFLLAVMAYDRFVAICNPLLYTVAMSQRLCALLVAGSYLWGMFGPLVLLCYALRL
NFSGPNVINHFFCEYTALISVSGSDILIPHLLLFSFATFNEMCTLLIILTSYVFIFVTVLKIRSVSGRHKAFSTWASHLTAITI
FHGTILFLYCVPNSKNSRQTVKVASVFYTVVNPMLNPLIYSLRNKDVKDAFWKLIHTQVPFH

Unless specifically addressed as GPCR2a or GPCR2b, any reference to GPCR2 is assumed to encompass all variants. Residue differences between any GPCRX variant sequences herein are written to show the residue in the "a" variant, the residue position with respect to the "a" variant, and the residue in the "b" variant. GPCRX residues in all following sequence alignments that differ between the individual GPCRX variants are highlighted in black and marked with the (o) symbol above the variant residue in all alignments herein. For

example, the GPCR2 nucleic acid sequences differ at the following two positions: C648T and C922T. The GPCR2 polypeptides differ only at one residue, namely P290L.

In a BLASTN search of sequence databases, it was found, for example, that the GPCR2a nucleic acid sequence has 471 of 648 bases (72%) identical to *Rattus norvegicus* taste bud receptor protein (SEQ ID NO:30), as shown in Table 2E. The BLASTN alignment shown in Table 2E result from a search utilizing the nucleotide sequence for GPCR2a. The residue that differs between GPCR2a and GPCR2b is highlighted in black and marked with the (o) symbol.

Table 2E. BLASTN of GPCR2 against rat taste bud receptor protein.

```
>gb:GENBANK-ID:RNU50948|acc:U50948 Rattus norvegicus taste bud receptor protein TB
    567 (TB 567) gene, complete cds - Rattus norvegicus, 1299 bp. (SEQ ID NO:30)
Score = 1221 (183.2 bits), Expect = 3.3e-49, P = 3.3e-49
Identities = 591/940 (62%), Positives = 591/940 (62%), Strand = Plus / Plus

Query:  523 TTTGTTGATTTTTGTTACTCTTCCATTGTCACTCCCAAGCTGCTTGAGAACTTGGTAATG 582
           ||||||||||| ||||| ||  |||   | || || || ||||| ||||||||||  ||
Sbjct:    2 TTTGTTGATTTCTGTTATTCCACCACAATTACACCAAAACTGCTGGAGAACTTGGTTGTG 61

Query:  583 GCAGATAAAAGCATCTTCTACTTTAGCTGCATGATGCAGTACTTCCTGTCCTGCACTGCT 642
           | ||| | || ||||| || |     | ||||| ||||| | |||| |  |||| |    |
Sbjct:   62 GAAGACAGAATCATCTCCTTCACAGGATGCATCATGCAATTCTTCTTTGCCTGTATATTT 121

Query:  643 GTGGTGACAGAGTCTTTCTTGCTGGCAGTGATGGCCTATGACCGCTTTGTGGCCATCTGC 702
           ||||||||||  | ||| |||||||| |||||| |||||| | ||||||||  | ||
Sbjct:  122 GTGGTGACAGAAACATTCATGCTGGCAGCGATGGCTTATGACAGATTTGTGGCAGTGTGT 181

Query:  703 AATCCTCTGCTTTATACAGTGGCCATGTCACAGAGGCTCTGTGCCCTGCTGGTGGCTGGG 762
           || |||||||||| ||||| || ||||| |||||||| ||  || || | |||||||
Sbjct:  182 AACCGTCTGCTTTACACAGTTGCAATGTCCCAGAGGCTTTGCTCCTTGTTAGTGGCTGCA 241

Query:  763 TCATATCTCTGGG-GCAT-GTTTGGCCCCTTGG-TACTCCTTTGTTATGCTCTCCGGTTA 819
           ||||| ||||| || |  | |||||  || |    ||| | |  || || |  |  |||
Sbjct:  242 TCATA-CTCTTGGAGTTTAGTTTGTTCCTTAACATACACATACTTTCTGTTGACT--TTA 298

Query:  820 AACTTCTCT-GGACCTAATGTAATCAACCACTTCTTTTGTGAGTATACTGCTCTCATCTC 878
              || | | |||| |||  | || ||  ||||  | ||||||  |  ||||  |  |  |
Sbjct:  299 TCTTTTTGTAGGAC-TAACTTCATTAATAACTTTGTCTGTGAGCACGCTGCCATTGTTGC 357
                                                o
Query:  879 TGTGTCTGGCTCTGATATACT-CATCCCCCACCTGCTGCTTTTCAGCTTC-GCCACCTTC 936
           ||||||  |||||||     || |||   |||   | |  |||| || ||| || || |||
Sbjct:  358 TGTGTCCTGCTCTGACCC-CTACATGAGCCAGAAGGTCATTTT-AGTTTCTGCAACATTC 415

Query:  937 AATGAGATGTGTACACTACTGATCATCCTCACTTCCTATGTTTTCATTTTTGTGACTGTA 996
           ||||| ||  | |  ||  | ||||| |||||||||||||| |||||||||| | |||||
Sbjct:  416 AATGAAATAAGCAGCCTGGTAATCATTCTCACTTCCTATGCTTTCATTTTTATCACTGTC 475

Query:  997 CTAAAAATCCGTTCTGTTAGTGGGCGCCACAAAGCCTTCTCCACCTGGGCCTCCCACCTG 1056
            | || || | |||   | | |||||| | ||||| |||||||| || ||||||||||||
Sbjct:  476 ATGAAGATGCCTTCCACTGGGGGGCGCAAGAAAGCGTTCTCCACGTGTGCCTCCCACCTG 535

Query: 1057 ACTGCTATCACCATCTTCCATGGGACCATCCTTTTCCTTTACTGTGTACCCAACTCCAAA 1116
           || || || ||||| |||||||||| |||||||| || |||||||| || |||||||||
Sbjct:  536 ACCGCCATTACCATTTTCCATGGGACTATCCTTTTTCTCTACTGTGTTCCTAACTCCAAA 595

Query: 1117 AACTCTCGGCAAACAGTCAAAGTGGCCTCTGTATTTTACACAGTTGTCAACCCC 1170
           |  ||  |||  |  ||||| |||||||||| |||||||||| ||||  |||
Sbjct:  596 AGTTCATGGCTCATGGTCAAGGTGGCCTCTGTCTTTTACACAGTGGTCATTCCC 649
```

The GPCR2 nucleic acid sequence has homology to two regions of the *Homo sapiens* olfactory receptor ("OR5D3") gene, as shown in Table 2F. OR5D3 residues 437-644 (SEQ ID NO:31) has 168 of 208 bases (80%) identical to GPCR2, with an E value of $4x10^{-17}$. OR5D3 residues 121-219 (SEQ ID NO:32) has 82 of 99 bases (82%) identical to GPCR2, with an E value of $6x10^{-7}$.

Table 2F. BLASTN of GPCR2 against the OR5D3 gene

```
>gb|AF065860.1|AF065860 Homo sapiens olfactory receptor (OR5D3) gene, partial cds
   Length = 649;  Score = 95.6 bits (48), Expect = 4e-17;
   Identities = 168/208 (80%); Strand = Plus / Plus

Query: 696 atcatcctcacttcctatgttttcatttttgtgactgtactaaaaatccgttctgttagt 755
           ||||| |||||||||||||| |||||||||| | ||||| | || || | |||   | |
Sbjct: 437 atcattctcacttcctatgctttcatttttatcactgtcatgaagatgccttccactggg 496

Query: 756 gggcgccacaaagccttctccacctgggcctcccacctgactgctatcaccatcttccat 815
           |||||| | ||||| |||||||| || |||||||||||||| || || ||||| ||||||
Sbjct: 497 gggcgcaagaaagcgttctccacgtgtgcctcccacctgaccgccattaccattttccat 556

Query: 816 gggaccatccttttcctttactgtgtacccaactccaaaaactctcggcaaacagtcaaa 875
           ||||| |||||||| || |||||||| || |||||||||| || ||| | |||||
Sbjct: 557 gggactatccttttctctactgtgttcctaactccaaaagttcatggctcatggtcaag 616

Query: 876 gtggcctctgtattttacacagttgtca 903
           |||||||||||| |||||||||||| ||||
Sbjct: 617 gtggcctctgtcttttacacagtggtca 644   (SEQ ID NO:31)


Score = 61.9 bits (31), Expect = 6e-07
 Identities = 82/99 (82%) Strand = Plus / Plus

Query: 380 tgtggtgacagagtctttcttgctggcagtgatggcctatgaccgctttgtggccatctg 439
           ||||||||||||  | ||| ||||||||| |||||| |||||| | |||||||| | ||
Sbjct: 121 tgtggtgacagaaacattcatgctggcagcgatggcttatgacagatttgtggcagtgtg 180

Query: 440 caatcctctgctttatacagtggccatgtcacagaggct 478
            || |||||||||| ||||| || ||||| ||||||||
Sbjct: 181 taaccctctgctttacacagttgcaatgtcccagaggct 219  (SEQ ID NO:32)
```

The full GPCR2 amino acid sequence has 159 of 306 amino acid residues (51 %) identical to, and 214 of 306 residues (69 %) positive with, the 314 amino acid residue proteins from *Homo sapiens* Olfactory Receptor-like protein OLF1 (ptnr: SPTREMBL-ACC: Q13606) (SEQ ID NO:33) (Table 2G). The residue that differs between GPCR2a and GPCR2b is highlighted in black and marked with the (o) symbol.

Table 2G. BLASTX of GPCR2 against OR-like Protein OLF1

```
>ptnr:SWISSPROT-ACC:Q13606 OLFACTORY RECEPTOR-LIKE PROTEIN OLF1 - Homo sapiens
(Human), 314 aa.  (SEQ ID NO:33)
Score = 814 (286.5 bits), Expect = 1.6e-80, P = 1.6e-80
Identities = 159/306 (51%), Positives = 214/306 (69%), Frame = +1

Query: 6   RNLSMEPTFALLGFTDYPKLQIPLFLVFLLMYVITVVGNLGMIIIIKINPKFHTPMYFFL 65
           || ++   | ||||   |+||| |||+|| +| | ++||+|++++|+|+|   |||||||
Sbjct: 6   RNYTLVTEFILLGFPTRPELQIVLFLMFLTLYAIILIGNIGLMLLIRIDPHLQTPMYFFL 65

Query: 66  SHLSFVDFCYSSIVTPKLLENLVMADKSIFYFSCMMQYFLSCTAVVTESFLLAVMAYDRF 125
           |+|||||| || | + ||+| | +  +||| |+ | +|++  ||   ||||+|| |||||+
```

```
Sbjct: 66  SNLSFVDLCYFSDIVPKMLVNFLSENKSISYYGCALQFYFFCTFADTESFILAAMAYDRY 125

Query: 126 VAICNPLLYTVAMSQRLCALLVAGSYLWGMFGPLVLLCYALRLNFSGPNVINHFFCEYTA 185
           |||||||||||| ||+ +|  |+  |||  |     ||   +|  | +   ||||||||+
Sbjct: 126 VAICNPLLYTVVMSRGICMRLIVLSYLGGNMSSLVHTSFAFILKYCDKNVINHFFCDLPP 185

Query: 186 LISVSGSDILIPHLLLFSFATFNEMCTLLIILTSYVFIFVTVLKIRSVSGRHKAFSTWAS 245
           |+ +| +|  |   || ++ +  |+   +||+ || || ++|||||| ||| | ||| ||
Sbjct: 186 LLKLSCTDTTINEWLLSTYGSSVEIICFIIIIISYFFILLSVLKIRSFSGRKKTFSTCAS 245

Query: 246 HLTAITIFHGTILFLYCVPNSKNSRQTVKVASVFYTVVNPMLNPPIYSLRNKDVKDAFWK 305
           |||++||+ ||+||+|  |+  |  | |+ |||||+  |+||| ||||||||||||  |
Sbjct: 246 HLTSVTIYQGTLLFIYSRPSYLYSPNTDKIISVFYTIFIPVLNPLIYSLRNKDVKDAAEK 305

Query: 306 LIHTQV 311
           ++ ++|
Sbjct: 306 VLRSKV 311
```

The full amino acid sequence of the GPCR2 protein of the invention has 152 of 301 amino acid residues (50%) identical to, and 207 of 301 residues (68%) positive with, the 312 amino acid residue proteins from *Gallus gallus* olfactory receptor 4 (ptnr: SPTREMBL-ACC: CAA64370.1) (SEQ ID NO:34) (Table 2H). The residue that differs between GPCR2a and GPCR2b is highlighted in black and marked with the (o) symbol.

Table 2H. BLASTX of GPCR2 against OR-4

```
>emb|CAA64370.1| (X94744) olfactory receptor 4 [Gallus gallus]; (SEQ ID NO:34), 312
aa
Statistics for GPCR2a: Score =  304 bits (779), Expect = 8e-82
                       Identities = 152/301 (50%), Positives = 207/301 (68%)
Statistics for GPCR2b: Score =  320 bits (821), Expect = 1e-86
                       Identities = 160/306 (52%), Positives = 215/306 (69%)

Query: 7   NLSMEPTFALLGFTDYPKLQIPLFLVFLLMYVITVVGNLGMIIIIKINPKFHTPMYFFLS 66
           | ++   | |+| +|+||++  ||+||||+||||  ||||+|||+|+ +|+ || ||||||
Sbjct: 5   NHTLASEFILVGLSDHPKMKAALFVVFLLIYVITFQGNLGIIILIQGDPRLHTSMYFFLS 64

Query: 67  HLSFVDFCYSSIVTPKLLENLVMADKSIFYFSCMMQYFLSCTAVVTESFLLAVMAYDRFV 126
            || || |+||++ |+ | | +   ++| +  |  | |     | || ||||||||||+|
Sbjct: 65  SLSVVDICFSSVIAPRTLVNFLSERRTISFTGCTGQTFFYIVFVTTECFLLAVMAYDRYV 124

Query: 127 AICNPLLYTVAMSQRLCALLVAGSYLWGMFGPLVLLCYALRLNFSGPNVINHFFCEYTAL 186
           |||||||+  |++| |  || |||+ |+   ++   + +|| | | |+|||||+   |
Sbjct: 125 AICNPLLYSTIMTRRQCMQLVVGSYIGGILNAIIQTTFIIRLPFCGSNIINHFFCDVPPL 184

Query: 187 ISVSGSDILIPHLLLFSFATFNEMCTLLIILTSYVFIFVTVLKIRSVSGRHKAFSTWASH 246
           +++| +   |  ++|||  |   |+ |+  || ||+||   +|+|||  || || || |||
Sbjct: 185 LALSLASTYISEMILFSLAGIIELSTVTSILVSYIFISCAILRIRSAEGRQKALSTCASH 244

Query: 247 LTAITIFHGTILFLYCVPNSKNSRQTVKVASVFYTVVNPMLNPPIYSLRNKDVKDAFWKL 306
           |||+|+ +|| +| |  |+|  |  | || ||||||| ||||| ||||||++||  |  ++
Sbjct: 245 LTAVTLLYGTTIFTYLRPSSSYSLNTDKVVSVFYTVVIPMLNPLIYSLRNQEVKGALSRV 304

Query: 307 I 307
           +
Sbjct: 305 V 305
```

A multiple sequence alignment is given in Table 2I, with the GPCR2 protein of the invention being shown on line 1, in a ClustalW analysis comparing GPCR2 with related protein sequences.

Table 2I.-Information for the ClustalW proteins:

1. Novel_Human_OLF, *i.e.* GPCR2, SEQ ID NO:4
2. *Homo sapiens* OLF, ptnr-SWISSPROT Acc # Q13606, SEQ ID NO:33
3. *Gallus gallus* OLF, ptnr-SWISSPROT Acc # P37070, SEQ ID NO:35
4. *Rattus norvegicus* OLF, Acc # Q63395, SEQ ID NO:36

```
GPCR2          MMMVLRNLSMEPTFALLGFTDYEKLQIPLFLVFLLMYVITVVGNLGMIIIIKINEKFHTP
HUMAN_OLF      MEFTDRNYTLVTEFILLGFPTRPELQIVLFLMFLTLYAIILIGNIGLMLLIRIDPHLQTP
CHICK_OLF4     --MASGNCTTPTTFILSGLTDNPGLQMPLFMVFLAIYAITLLTNLGLIALISVDLHLQTP
RAT_OLF        -MMGTGNHSAVVVFVLVGLTKQPELLLPLFFLFLVIYVLTVVGNLGMILLIIVSPLLHTP

GPCR2          MYFFLSHLSFVDFCYSSIVTPKLLENLVMADKSIFYFSCMMQYFLSCTAVVTESFLLAVM
HUMAN_OLF      MYFFLSNLSFVDLCYFSDIVPKMLVNFLSENKSISYYGCALQFYFFCTFADTESFILAAM
CHICK_OLF4     MYIFLQNLSFTDAAYSTVITPKMLATFLEERKTISYIGCILQYFSFVLLTVTESLLLAVM
RAT_OLF        MYYFLSSLSFVDLCYSTVITPKMLVNFLGKKNFITYSECMAQFFFFAIFVVTEGYLLTVM

GPCR2          AYDRFVAICNPLLYTVAMSQRLCALLVAGSYLWGMFGPLVLLCYALRLNFSGPNVINHFF
HUMAN_OLF      AYDRYVAICNPLLYTVVMSRGICMRLIVLSYLGGNMSSLVHTSFAFILKYCDKNVINHFF
CHICK_OLF4     AYDRYVAICKPLLYPSIMTKAVCWRLVKGLYSLAFLNSLVHTSGLLKLSFCSSNVVNHFF
RAT_OLF        AYDRYVAICRPLLYNVIMSSRICSLLVLVAFSLGLFSAVVHTSAMMNLSFCKSYITSHYF

GPCR2          CEYTALISVSGSDILIPHLLLFSFATFNEMCTLLIILTSYVFIFVTVLKIRSVSGRHKAF
HUMAN_OLF      CDLPPLLKLSCTDTTINEWLLSTYGSSVEIICFIITILSYFFILLSVLKIRSFSGRKKTF
CHICK_OLF4     CDNSPLFQISSSSTTLNELLVFIFGSLFAMSSIITILLISYVFIILTVVRIRSKDGKYKAF
RAT_OLF        CDALPLLKLACSNTHLNELLIFIIGGLNTLVPTLAVAISYVFIFCSILRIRSSEGRSKAF

GPCR2          STWASHLTAITIFHGTILFLYCVPNSKNSRQTVKVASVFYTVVNPMLNPFIYSLRNKDVK
HUMAN_OLF      STCASHLTSVTIYQGTLLFIYSRPSYLYSPNTDKIISVFYTIFIPVLNPLIYSLRNKDVK
CHICK_OLF4     STCTSHLMAVSLFHGTVIFMYLRPVKLFSLDTDKIASLFYTVVIPMLNPLIYSWRNKEVK
RAT_OLF        GTCSSHLMAVGIFFGSITFMYLKPSSSNSLEQEKVSSVFYTTVIPMLNPLIYSLRNKDVK

GPCR2          DAFWKLIHTQVPFH
HUMAN_OLF      DAAEKVLRSKVDSS
CHICK_OLF4     DALRRVIATNVWIH
RAT_OLF        KALGRFSVRS----
```

DOMAIN results for GPCR2 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 2J with the statistics and domain description. The 7tm_1, a seven transmembrane receptor (rhodopsin family), was shown to have two segments with significant homology to GPCR2. An alignment of GPCR2 with residues 1-170 (SEQ ID NO:29) and residues 310-377 (SEQ ID NO:37) of 7tm_1 are shown in Table 2J.

Table 2J.-DOMAIN results for GPCR2

```
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID
NO:29)
       Length = 377;  Score = 83.2 bits (204), Expect = 2e-17
Query:  43   GNLGMIIIIKINPKFHTPMYFFLSHLSFVDFCYSSIVTPKLLENLVMADKSIFYFSCMMQ  102
Sbjct:  1    GNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF  60
             **: : : :       *   : :  *:  *   :::* * ::   *: :       * :

Query:  103  YFLSCTAVVTESFLLAVMAYDRFVAICNPLLYTVAMSQRLCALLVAGSYLWGMFGPLVLL  162
Sbjct:  61   VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVM----------IAI  108
               *           *  :: **: *:  *:**    * :    ::           : :

Query:  163  CYALRLNFSGPNVINHFFCEYTALISVSGSDILIPHLLLFSFATFNEMCTLLIILTSYVF  222
Sbjct:  109  VWVLSFTISCPMLF------GLNNTDQNECIIANPAFVVYSSIVSF-YVPFIVTLLVYIK  161
              : *    * * :             :   * *  :::*          :: *  *:
```

```
Query:  223  IFVTVLKIR  231
Sbjct:  162  IYIVLRRRR  170
             *:: : : *

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID
NO:37)
Length = 377
             Score = 35.8 bits (81), Expect = 0.003
Query:  226  TVLKIRSVSGRHKAFSTWASHLTAITIFHGT-ILFLYCVPNSKNSRQTVKVASVFYTVVN  284
Sbjct:  310  SRRKLSQQKEKKATQMLAIVLGVFIICWLPFFITHILNIHCDCNIPPVLYSAFTWLGYVN  369
             :  *:     :            *  :    *  :  :    *    : *  :   **
                 o
Query:  285  PMLNPLIY  292
Sbjct:  370  SAVNPIIY  377
               :**:**
```

The nucleic acids and proteins of GPCR2 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor-like protein may be useful in gene therapy, and the olfactory receptor-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Other GPCR-1 diseases and disorders are contemplated.

The novel nucleic acid encoding GPCR2, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. This novel protein also has immense value in development of powerful assay system for functional analysis.

GPCR3

An additional GPCR-like protein of the invention, referred to herein as GPCR3, is an Olfactory Receptor ("OR")-like protein. The GPCR3 nucleic acid of 1001 nucleotides (also designated AP001112_A) is shown in Table 3A. An ORF was identified beginning with an ATG initiation codon at nucleotides 12-14 and ending with a TAA codon at nucleotides 945-47. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 3A, and the start and stop codons are in bold letters.

Table 3A. GPCR3 Nucle tide Sequence (SEQ ID NO:7)

CGAAGGAAATTATGAGAAGAAACTGCACGTTGGTGACTGAGTTCATTCTCCTGGGACTGACCAGTCGCCGGGAATTACAAATTC
TCCTCTTCACGCTGTTTCTGGCCATTTACATGGTCACGGTGGCAGGGAACCTTGGCATGATTGTCCTCATCCAGGCCAACGCCT
GGCTCCACATGCCCATGTACTTTTTCCTGAGCCACTTATCCTTCGTGGATCTGTGCTTCTCTTCCAATGTGACTCCAAAGATGC
TGGAGATTTTCCTTTCAGAGAAGAAAAGCATTTCCTATCCTGCCTGTCTTGTGCAGTGTTACCTTTTTATCGCCTTGGTCCATG
TTGAGATCTACATCCTGGCTGTGATGGCCTTTGACCGGTACATGGCCATCTGCAACCCTCTGCTTTATGGCAGCAGAATGTCCA
AGAGTGTGTGCTCCTTCCTCATCACGGTGCCTTATGTGTATGGAGCGCTCACTGGCCTGATGGAGACCATGTGGACCTACAACC
TAGCCTTCTGTGGCCCCAATGAAATTAATCACTTCTACTGTGCGGACCCACCACTGATTAAGCTGGCTTGTTCTGACACCTACA
ACAAGGAGTTGTCAATGTTTATTGTGGCTGGCTGGAACCTTTCTTTTTCTCTCTTCATCATATGTATTTCCTACCTTTACATTT
TCCCTGCTATTTTAAAGATTCGCTCTACAGAGGGCAGGCAAAAAGCTTTTTCTACCTGTGGCTCCCATCTGACAGCTGTCACTA
TATTCTATGCAACCCTTTTCTTCATGTATCTCAGACCCCCCTCAAAGGAATCTGTTGAACAGGGTAAAATGGTAGCTGTATTTT
ATACCACAGTAATCCCTATGCTGAACCTTATAATTTATAGCCTTAGAAATAAAAATGTAAAAGAAGCATTAATCAAAGAGCTGT
CAATGAAGATATACTTTTCTTAAAAATCAGTATTCTTTTGGTTTCTAAAGCCCTTCCTAGACTTTTTTCTTTAGCTG

The GPCR3 polypeptide (SEQ ID NO:8) encoded by SEQ ID NO:7 is 311 amino acid residues and is presented using the one-letter code in Table 3B.

Table 3B. Encoded GPCR3 protein sequence (SEQ ID NO:8).

MRRNCTLVTEFILLGLTSRRELQILLFTLFLAIYMVTVAGNLGMIVLIQANAWLHMPMYFFLSHLSFVDLCFSSNVTPKMLEIF
LSEKKSISYPACLVQCYLFIALVHVEIYILAVMAFDRYMAICNPLLYGSRMSKSVCSFLITVPYVYGALTGLMETMWTYNLAFC
GPNEINHFYCADPPLIKLACSDTYNKELSMFIVAGWNLSFSLFIICISYLYIFPAILKIRSTEGRQKAFSTCGSHLTAVTIFYA
TLFFMYLRPPSKESVEQGKMVAVFYTTVIPMLNLIIYSLRNKNVKEALIKELSMKIYFS

In a search of sequence databases, it was found, for example, that the GPCR3 nucleic acid sequence has 609 of 923 bases (65%) identical to a and 609/923 bases (65%) positive with *Pan troglodytes* species Olfactory Receptor OR93 gene (SEQ ID NO:38), as shown in Table 3C.

Table 3C. BLASTN of GPCR3 against Chimpanzee OR93 gene

```
>gb:GENBANK-ID:AF045577|acc:AF045577 Pan troglodytes olfactory receptor OR93Ch
        (OR93) gene, complete cds - Pan troglodytes, (SEQ ID NO:38), 989 bp
Score = 1405 (210.8 bits), Expect = 2.1e-57, P = 2.1e-57
Identities = 609/923 (65%), Positives = 609/923 (65%), Strand = Plus / Plus

Query:    20 AAACTGCACGTTGGTGACTGAGTTCATTCTCCTGGGACTGACC-AGTCGCCGGGAATTAC 78
             ||||| |||   ||| || || |||||| ||   ||  |||   |    ||   | || |
Sbjct:    12 AAACTACACAAAGGTCACCGAATTCATTTTCACAGGCTTGAATTACAATCCTC-AGTTGC 70

Query:    79 AAATTCTCCTCTTCACGCTGTTTCTGGCCATTTAC-ATGGTCA-CGGTGGCAG-GGAACC 135
             |  | ||||||||   || |||||| | | || | ||| ||| |  ||  |  |||| |
Sbjct:    71 AGGTCTTCCTCTTCCTACTCTTTCTGACAACTTTCTATG-TCATCAATGTAACTGGAAAC 129

Query:   136 TTGGCA-TGATTGTCCTCATCCAGGCCAACGCCTGGCTCCACATGCCCATGTACTTTTTC 194
             |||| | |||||||||| ||||    | |  || | || |||| ||||||||||||||
Sbjct:   130 TTGGGAATGATTGTCCTTATCCGAATCGATTCCCGCCTTCACACACCCATGTACTTTTTC 189

Query:   195 CTGAGCCACTTATCCTTCGTGGATCTGTGCTTCTCTTCCAATGTGACTCCAAAGATGCTG 254
             || |||||| | ||||| |||||  | |||||||| ||   |||||  || ||||||||
Sbjct:   190 CTCAGCCACCTGTCCTTTGTGGACATCTGCTTCTCCTCAGTTGTGAGCCCCAAGATGCTC 249

Query:   255 GA-GATTTTCCTTTCAGAGAAGAAAAGC-ATTTCCTATCCT-GCCTGT-CTT-GT-GCAG 308
             .    || ||  ||||  ||  || ||||| ||||| | |||| | |||| ||| |  ||||
Sbjct:   250 ACTGACTT--CTTTGTGAAGAGGAAAGCCATTTCTT-TCCTTGGCTGTGCTTTGCAGCAG 306

Query:   309 TGTTACCTTTTTATCGCCTTGGTCCATGTTGAGATCTACATCCTGGCTGTG-ATGGCCTT 367
             || | | |||   |  | |||   || |  |||   | | || |||| ||  |||||||
Sbjct:   307 TGGTTC-TTTGGGTT-CTTTGTGGCA-GCAGAGTGTTTCCTCTTGGC-GTCCATGGCCTA 362

Query:   368 TGACCGGTACATGGCCATCTGCAACCCTCTGCTTTATGGCAGCAGA-ATGTCCAAGAGTG 426
             |||||| ||  |||||||||| |||||  || | ||   |||  |  |||||| ||||
```

```
Sbjct:   363 TGACCGCTATGTGGCCATCTGTAACCCATTGTTATACT-CAGTTGCTATGTCCCAGAGGC 421

Query:   427 TGTGC-TCCTTCCTCATCACGGTGCCTTATGTGTATGGAGCGC-TCACTGGCCTGATGGA 484

Sbjct:   422 TCTGCATCCAGC-TAGTGGTGGGTCCCTATGTCATTGGA-CTCATGAATACCATGACTCA 479

Query:   485 GAC--CATGTGGACCTACAACCTAGCCTTCTGTGGCCCCAATGAAATTAATCACTTCTAC 542

Sbjct:   480 CACAACAAATGCATTT-CGTC-TCCCTTTTTGTGGCCCTAATGTCATCAATCATTTCTTC 537

Query:   543 TGTGCGGACCCACCACTGA-TTAAGCTGGCTTGTTCTGACACCTACAACAAGGAGTTGTC 601

Sbjct:   538 TGTGATATGTCCCC-CTTACTTTCCCTTGTATGTGCTGATACCAGGCTCAATAAGTTGGC 596

Query:   602 AATGTTTATTGTGGCTGG--CTG-GAACCT-TTCTTTT-TCTCTCTTCATCATATGTATT 656

Sbjct:   597 AGTTTTCATCGTGGCTGGAGCTGCGGGAGTCTTCAGTGGTCTGACT--ATCCT--G-ATT 651

Query:   657 TCCTACCTTTACATTTTCCCTGCTATTTTAAAGATTCGCTCTACAGAGGGCAGGCAAAAA 716

Sbjct:   652 TCCTACATTTACATCCTCATGGCCATCCTGAGGATCCGCTCTGCTGATGGGAGGTGCAAA 711

Query:   717 GCTTTTTCTACCTGTGGCTCCCATCTGACAGCTGTCACTATATTCTATGCAACCCTTTTC 776

Sbjct:   712 ACCTTTTCTACTTGCTCTTCTCACCTGACAGCTGTTTTCATCTTGTATGGTACCCTTTTC 771

Query:   777 TTCATGTATCTCAGACCCCCCTCAAAGGAATCTGTTGAACAGGGTAAAATGGTAGCTGTA 836

Sbjct:   772 TTTATTTATGTACGTCCTAGTGCAAGCTTCTCCCTGGATCTCAATAAATTAGTGTCTGTG 831

Query:   837 TTTTATACCACAGTAATCCCTATGCTGAACCTTATAATTTATAGCCTTAGAAATAAAAAT 896

Sbjct:   832 TTTTACACAGCAGTGATTCCTATGTTGAACCCACTTATCTACAGCTTGAGAAACAAGGAA 891

Query:   897 GTAAAAGAAGC-ATTAATCAAAGAGCTGTCAATGAAGATATACTTTT 942

Sbjct:   892 GTCAAAGATGCCATCCA-CAG-GA-CTGTCACTCA-GAGGAAGTTTT 934
```

The BLASTN alignment shown in Table 3D indicates that two fragments of GPCR3 have homology to fragments of *Mus musculus* olfactory receptor 4 cluster, gene 3 ("Olfr4-3") (GENBANK-ID: NM_013728.1). Residues 827-907 (SEQ ID NO:39) and residues 163-210 (SEQ ID NO:40) of the Olfr4-3 gene are shown below.

Table 3D-BLASTN of GPCR3 against Olfr4-3

```
>ref|NM_013728.1| Mus musculus olfactory receptor 4 cluster, gene 3 (Olfr4-3), mRNA
          Length = 957 (SEQ ID NO:39)
 Score = 58.0 bits (29), Expect = 9e-06
 Identities = 68/81 (83%)
 Strand = Plus / Plus

Query: 835 tattttataccacagtaatccctatgctgaaccttataatttatagccttagaaataaaa 894

Sbjct: 827 tattttataccactgttatccctatgttgaacccattcatttatagcctgagaaataagg 886

Query: 895 atgtaaaagaagcattaatca 915

Sbjct: 887 aagtcaaagatgcattaatca 907

>ref|NM_013728.1| Mus musculus olfactory receptor 4 cluster, gene 3 (Olfr4-3), mRNA
          Length = 957 (SEQ ID NO:40)
 Score = 56.0 bits (28), Expect = 4e-05
 Identities = 43/48 (89%)
 Strand = Plus / Plus
```

```
Query: 171 ctccacatgcccatgtacttttttcctgagccacttatccttcgtggat 218
           ||||||||  |||||||||||||||  ||  ||||||||  |||||  ||||||
Sbjct: 163 ctccacatccccatgtacttttttctcagccacttgtcctttgtggat 210
```

The full GPCR3 amino acid sequence has 166 of 305 amino acid residues (54%) identical to, and 214 of 305 residues (70%) positive with, the 314 amino acid residue OR93CH protein from *Pan troglodytes* (ptnr: SPTREMBL-ACC: O77756) (SEQ ID NO:41) (Table 3E).

Table 3E. BLASTX of GPCR3 against OR93CH - (SEQ ID NO:41)

```
>SPTREMBL-ACC:O77756 OLFACTORY RECEPTOR OR93CH - Pan troglodytes (Chimpanzee), 314
aa.
Score = 832 (292.9 bits), Expect = 3.6e-82, P = 3.6e-82
Identities = 166/305 (54%), Positives = 214/305 (70%), Frame = +3

Query: 4   NCTLVTEFILLGLTSRRELQILLFTLFLA-IYMVTVAGNLGMIVLIQANAWLHMPMYFFL 62
           | | |||||  ||    +||+ || |||   |++ | ||||||||+ ++ || ||||||
Sbjct: 5   NYTKVTEFIFTGLNYNPQLQVFLFLLFLTTFYVINVTGNLGMIVLIRIDSRLHTPMYFFL 64

Query: 63  SHLSFVDLCFSSNVTPKMLEIFLSEKKSISYPACLVQCYLFIALVHVEIYILAVMAFDRY 122
           |||||||+|||| |+|||| | ++|+||+ | +| + |  | | ++|| ||+|||
Sbjct: 65  SHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMAYDRY 124

Query: 123 MAICNPLLYGSRMSKSVCSFLITVPYVYGALTGLMETMWTYNLAFCGPNEINHFYCADPP 182
           +|||||||  ||+ +|  |+ ||| | +  +  |  + | ||||| ||||+|  |
Sbjct: 125 VAICNPLLYSVAMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGPNVINHFFCDMSP 184

Query: 183 LIKLACSDTYNKELSMFIVAGWNLSFSLFIICISYLYIFPAILKIRSTEGRQKAFSTCGS 242
           |+ | |+||  +|++|||||  ||  | |||+||  |||+||| +|| | |||| |
Sbjct: 185 LLSLVCADTRLNKLAVFIVAGAAGVFSGLTILISYIYILMAILRIRSADGRCKTFSTCSS 244

Query: 243 HLTAVTIFYATLFFMYLRPPSKESVEQGKMVAVFYTTVIPMLNLIIYSLRNKNVKEALIK 302
           ||||| | | ||||+|+|| +  |++  |+|+|||| |||||| +|||||| ||+|+ +
Sbjct: 245 HLTAVFILYGTLFFIYVRPSASFSLDLNKLVSVFYTAVIPMLNPLIYSLRNKEVKDAIHR 304

Query: 303 ELSMK 307
            ++ +
Sbjct: 305 TVTQR 309
```

The full amino acid sequence of the GPCR3 protein was also found to have 166 of 311 amino acid residues (53%) identical to, and 215 of 311 residues (68%) positive with, the 311 amino acid residue proteins from *Mus musculus* olfactory receptor 4 cluster, gene 3 (SEQ ID NO:42), shown in Table 3F.

Table 3F. BLASTX of GPCR3 against mouse OR4 cluster, gene 3

```
>ref|NP_038756.1| olfactory receptor 4 cluster, gene 3 [Mus musculus], derived from
 gb|AAF20365.1|AF146372_1 (AF146372) olfactory receptor OR912-93 [Mus musculus
domesticus]  (SEQ ID NO:42).
 Length = 318; Score =  321 bits (823), Expect = 6e-87
 Identities = 166/311 (53%), Positives = 215/311 (68%)

Query: 1   MRRNCTLVTEFILLGLTSRRELQILLFTLFLAIYMVTVAGNLGMIVLIQANAWLHMPMYF 60
           | || |+|||| |||| |||+|| || +|+ |+ ||||||+||  +  ||+||||
Sbjct: 2   MHRNQTVVTEFFFTGLTSSFHLQIVLFLTFLCVYLATLLGNLGMIILIHLDTRLHIPMYF 61

Query: 61  FLSHLSFVDLCFSSNVTPKMLEIFLSEKKSISYPACLVQCYLFIALVHVEIYILAVMAFD 120
           |||||||||  | || ++|||| +|| ||+ | +|  ||  |  | ++|| ||+|
Sbjct: 62  FLSHLSFVDACSSSVISPKMLSDMFVDKKVISFLGCAIQLCLFSQFVVTECFLLASMAYD 121

Query: 121 RYMAICNPLLYGSRMSKSVCSFLITVPYVYGALTGLMETMWTYNLAFCGPNEINHFYCAD 180
           ||+||| |||| ||+ ||  |+  ||  | ++ ++  +  + | +|||| ||||+|
```

```
Sbjct: 122 RYVAICKPLLYTLIMSQRVCVQLVIGPYSIGFISTMVHIISAFVLPYCGPNLINHFFCDL 181

Query: 181 PPLIKLACSDTYNKELSMFIVAGWNLSFSLFIICISYLYIFPAILKIRSTEGRQKAFSTC 240
           |++ |||++|   +  +|||||     ||  || +||+||   |||| | +||+||||||
Sbjct: 182 LPVLSLACANTQMNKRLLFIVAGILGVFSGIIILVSYVYIAITILKISSADGRRKAFSTC 241

Query: 241 GSHLTAVTIFYATLFFMYLRPPSKESVEQGKMVAVFYTTVIPMLNLIIYSLRNKNVKEAL 300
           ||||||+| | ||||+|+|| |  |++  |+|++|||||||||  |||||||.||+||
Sbjct: 242 SSHLTAVSILYGTLFFIYVRPSSSFSLDINKVVSLFYTTVIPMLNPFIYSLRNKEVKDAL 301

Query: 301 IKELSMKIYFS 311
           |+   +  +|
Sbjct: 302 IRTFEKQFCYS 312
```

A multiple sequence alignment is given in Table 3G, with GPCR3 being shown on line 4, in a ClustalW analysis comparing GPCR3 with related protein sequences.

Table 3G. Information for the ClustalW proteins:

1. *Hylobates lar* (Common Gibbon) OLF, SPTREMBL -Acc # O77758, SEQ ID NO:43
2. *Pan troglodytes* (Chimpanzee) OLF, SPTREMBL-Acc # O77756, SEQ ID NO:41
3. *Mus musculus* OLF, GENBANK-Acc # AAF20365, SEQ ID NO:42
4. Novel Human_OLF, GPCR3, SEQ ID NO:8

```
GIBBON_OLF      MANENYTKVTEFIFTGLNYNPQLQVFLFLLFLT-FYVISVTGNFGMIVLIRMDSRLHTPM
CHIMPANZEE_OLF  MANENYTKVTEFIFTGLNYNPQLQVFLFLLFLTTFYVINVTGNLGMIVLIRIDSRLHTPM
MOUSE_OLF       MMHRNQTVVTEFFFTGLTSSFHLQIVLFLTFLC-VYLATLLGNLGMIILIHLDTRLHIPM
GPCR3           -MRRNCTLVTEFILLGLTSRRELQILLFTLFLA-IYMVTVAGNLGMIVLIQANAWLHMPM

GIBBON_OLF      YFFLSHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMA
CHIMPANZEE_OLF  YFFLSHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMA
MOUSE_OLF       YFFLSHLSFVDACSSSVISPKMLSDMFVDKKVISFLGCAIQLCLFSQFVVTECFLLASMA
GPCR3           YFFLSHLSFVDLCFSSNVTPKMLEIFLSEKKSISYPACLVQCYLFIALVHVEIYILAVMA

GIBBON_OLF      YDRYVAICNPLLYSVFMSQRLCIQLVVGPYVIGLMNTMTHTTHAFRLPFCGLNVINHFFC
CHIMPANZEE_OLF  YDRYVAICNPLLYSVAMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGPNVINHFFC
MOUSE_OLF       YDRYVAICKPLLYTLIMSQRVCVQLVIGPYSIGFISTMVHIISAFVLPYCGPNLINHFFC
GPCR3           FDRYMAICNPLLYGSRMSKSVCSFLITVPYVYGALTGLMETMWTYNLAFCGPNEINHFYC

GIBBON_OLF      DMSPLLSIVCADTRLNKLAVFIMAGAVGVFSGLTILISYIYILMAILRIRSADGRCKTFS
CHIMPANZEE_OLF  DMSPLLSIVCADTRLNKLAVFIVAGAAGVFSGLTILISYIYILMAILRIRSADGRCKTFS
MOUSE_OLF       DLLPVLSLACANTQMNKRLLFIVAGILGVFSGIIILVSYVYTAITILKISSADGRRKAFS
GPCR3           ADPPLIKLACSDTYNKELSMFIVAGWNLSFSLFIICISYLYIFPAILKIRSTEGRQKAFS

GIBBON_OLF      TCSSHLTAVFILYGTLFFIYVRPSASFPLDLNKVVSVFYTAVIPMLNPLIYSLRNKEVKD
CHIMPANZEE_OLF  TCSSHLTAVFILYGTLFFIYVRPSASFSLDLNKLVSVFYTAVIPMLNPLIYSLRNKEVKD
MOUSE_OLF       TCSSHLTAVSILYGTLFFIYVRPSSSFSLDINKVVSLFYTTVIPMLNPFIYSLRNKEVKD
GPCR3           TCGSHLTAVTIFYATLFFMYLRPPSKESVEQGKMVAVFYTTVIPMLNLIIYSLRNKNVKE

GIBBON_OLF      AIHRTVTQRKFCKA------
CHIMPANZEE_OLF  AIHRTVTQRKFCKA------
MOUSE_OLF       ALIRTF-EKQFCYSLQDKIL
GPCR3           ALIKELSMKIYFS-------
```

DOMAIN results for GPCR3 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 3H with the statistics and domain description.

Table 3H.-DOMAIN results for GPCR3.

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID NO:29)

```
              Length = 377
              Score = 92.8 bits (229), Expect = 2e-20

Query: 40   GNLGMIVLIQANAWLHMPMYFFLSHLSFVDLCFSSNVTPKMLEIFLSEKKSISYPACLVQ  99
Sbjct: 1    GNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF  60
             **: : : :     *     : :  *:  **  :: * * :: : :  :    *    * :

Query: 100  CYLFIALVHVEIYILAVMAFDRYMAICNPLLYGSRM-SKSVCSFLITVPYVYGALTGLME  158
Sbjct: 61   VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVWVLSFTISCPM  120
               * : :    *  *  :: *** *:  *:** :*  **    : :* : :*

Query: 159  TMWTYNLAFCGPNEINHFYCADPPLIKLACSDTYNKELSMFIVAGWNLSFSLFIICISYL  218
Sbjct: 121  LFGLNNTDQNE------------------CIIANPAFVVYSSIVS--FYVPFIVTLLVYI  160
                  *                      *       :    :           :    *:

Query: 219  YIFPAILKIRSTEGRQK  235
Sbjct: 161  KIYIVLRRRRKRVNTKR  177
               *:   : : *      ::
```

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor-like protein may be useful in gene therapy, and the olfactory receptor -like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Other GPCR-related diseases and disorders are contemplated.

The novel nucleic acid encoding Olfactory receptor-like protein, and the Olfactory receptor-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. This novel protein also has immense value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders

GPCR4

GPCR4 is an Olfactory Receptor ("OR")-like protein, wherein two alternative novel GPCR4 nucleic acids and encoded polypeptides are disclosed.

The novel GPCR4a nucleic acid of 980 nucleotides (also referred to as AP001112_B) is shown in Table 4A. An ORF begins with an ATG initiation codon at nucleotides 19-21 and ends with a TAA codon at nucleotides 940-42. A putative untranslated region upstream from

the initiation codon and downstream from the termination codon is underlined in Table 4A, and the start and stop codons are in bold letters.

Table 4A. GPCR4a Nucleotide Sequence (SEQ ID NO:9)

TGACTTAGAATTCAGAAA**ATG**CTCAATTTCACCGATGTGACAGAGTTCATTCTTTTGGGGCTAACGAGCCGTCGAGAATGGCAA
GTTCTCTTCTTCATCATCTTTCTTGTGGTCTACATCATCACCATGGTGGGCAATATCGGCATGATGGTGTTAATCAAGGTCAGT
CCTCAGCTTAACAACCCCATGTACTTTTTCCTCAGTCACTTGTCATTTGTTGATGTGTGGTTTTCTTCCAATGTCACCCCTAAA
ATGTTGGAAAACCTGTTTTCAGATAAAAAAACAATTACTTATGCTGGTTGTTTAGTACAGTGTTTCTTCTTCATTGCTCTTGTC
CATGTGGAAATTTTTATTCTTGCTGCGATGGCCTTTGATAGATACATGGCAATTGGGAATCCTCTGCTTTATGGCAGTAAAATG
TCAAGGGTTGTCTGTATTCGACTGATTACTTTCCCTTACATTTATGGTTTTCTGACGAGTCTGGCAGCAACATTATGGACTTAC
GGCTTGTACTTCTGTGGAAAAATTGAGATCAACCATTTCTACTGTGCAGATCCACCTCTCATCAAAATGGCCTGTGCCGGGACC
TTTGTAAAAGAATATACAATGATCATACTTGCCGGCATTAACTTCACATATTCCCTGACTGTAATTATCATCTCTTACTTATTC
ATCCTCATTGCCATTCTGCGAATGCGCTCAGCAGAAGGAAGGCAGAAGGCCTTTTCCACATGTGGGTCCCATCTGACAGCTGTC
ATTATATTCTATGGTACTCTGATCTTCATGTATCTCAGACGTCCCACAGAGGAGTCTGTGGAGCAGGGGAAGATGGTGGCTGTG
TTCTATACCACAGTGATCCCCATGTTGAATCCCATGATCTACAGTCTGAGGAACAAGGATGTGAAAAAGGCCATGATGAAAGTG
ATCAGCAGATCATGTTAAACAAAATAAAATCAAATTTGATTTAATTTTATCTTCTA

The GPCR4a protein encoded by SEQ ID NO:9 has 307 amino acid residues and is presented using the one-letter code in Table 4B. The Psort profile for GPCR4 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a peptide is between amino acids 39 and 40, *i.e.*, at the dash in the amino acid sequence MVG-NIG, based on the SignalP result.

Table 4B. Encoded GPCR4a protein sequence (SEQ ID NO:10).

MLNFTDVTEFILLGLTSRREWQVLFFIIFLVVYIITMVGNIGMMVLIKVSPQLNNPMYFFLSHLSFVDVWFSSNVTPKMLENLF
SDKKTITYAGCLVQCFFFIALVHVEIFILAAMAFDRYMAIGNPLLYGSKMSRVVCIRLITFPYIYGFLTSLAATLWTYGLYFCG
KIEINHFYCADPPLIKMACAGTFVKEYTMIILAGINFTYSLTVIIISYLFILIAILRMRSAEGRQKAFSTCGSHLTAVIIFYGT
LIFMYLRRPTEESVEQGKMVAVFYTTVIPMLNPMIYSLRNKDVKKAMMKVISRSC

The target GPCR4a sequence was subjected an the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a library containing a wide range of cDNA species. The resulting amplicon was gel purified, cloned and sequenced to high redundancy. The 980 nucleotides of GPCR4b (also referred to as AC020597A) are provided in Table 4C. The resulting GPCR4b nucleotide sequence differs from that of GPCR4a at nine positions, namely A75G, A100G, C102T, C264T, T270A, C582T, A610C, T627C and T759C.

Table 4C. GPCR4b Nucleotide Sequence (SEQ ID NO:11)

TGACTTAGAATTCAGAAA**ATG**CTCAATTTCACCGATGTGACAGAGTTCATTCTTTTGGGGCTAACGAGCCGTCGGGAATGGCAA
GTTCTCTTCTTCATCGTTTTTCTTGTGGTCTACATCATCACCATGGTGGGCAATATCGGCATGATGTTGTTAATCAAGGTCAGT
CCTCAGCTTAACAACCCCATGTACTTTTTCCTCAGTCACTTGTCATTTGTTGATGTGTGGTTTTCTTCCAATGTCACCCCTAAA
ATGTTGGAAAATCTGTTATCAGATAAAAAAACAATTACTTATGCTGGTTGTTTAGTACAGTGTTTCTTCTTCATTGCTCTTGTC

CATGTGGAAATTTTTATTCTTGCTGCGATGGCCTTTGATAGATACATGGCAATTGGGAATCCTCTGCTTTATGGCAGTAAAATG
TCAAGGGTTGTCTGTATTCGACTGATTACTTTCCCTTACATTTATGGTTTTCTGACGAGTCTGGCAGCAACATTATGGACTTAC
GGCTTGTACTTCTGTGGAAAAATTGAGATCAACCATTTCTACTGTGCAGATCCACCTCTCATCAAAATGGCCTGTGCTGGGACC
TTTGTAAAAGAATATACAATGCTCATACTTGCCGGCATCAACTTCACATATTCCCTGACTGTAATTATCATCTCTTACTTATTC
ATCCTCATTGCCATTCTGCGAATGCGCTCAGCAGAAGGAAGGCAGAAGGCCTTTTCCACATGTGGGTCCCATCTGACAGCTGTC
ATCATATTCTATGGTACTCTGATCTTCATGTATCTCAGACGTCCCACAGAGGAGTCTGTGGAGCAGGGGAAGATGGTGGCTGTG
TTCTATACCACAGTGATCCCCATGTTGAATCCCATGATCTACAGTCTGAGGAACAAGGATGTGAAAAAGGCCATGATGAAAGTG
ATCAGCAGATCATGTTAAACAAAATAAAATCAAATTTGATTTAATTTTATCTTCTA

The GPCR4b protein encoded by SEQ ID NO:11 has 314 amino acid residues and a molecular weight of 35155.8 Daltons, as presented using the one-letter code in Table 4D. GPCR4a differs from GPCR4b at four residues, namely I28V, V45L, F84L and I198L. The signal peptide and Psort analyses for both GPCR4 variants are the same.

Table 4D. Encoded GPCR4b protein sequence (SEQ ID NO:12)

MLNFTDVTEFILLGLTSRREWQVLFFIVFLVVYIITMVGNIGMMLLIKVSPQLNNPMYFFLSHLSFVDVWFSSNVTPKMLENLL
SDKKTITYAGCLVQCFFFIALVHVEIFILAAMAFDRYMAIGNPLLYGSKMSRVVCIRLITFPYIYGFLTSLAATLWTYGLYFCG
KIEINHFYCADPPLIKMACAGTFVKEYTMLILAGINFTYSLTVIIISYLFILIAILRMRSAEGRQKAFSTCGSHLTAVIIFYGT
LIFMYLRRPTEESVEQGKMVAVFYTTVIPMLNPMIYSLRNKDVKKAMMKVISRSC

Unless specifically addressing GPCR4a or 4b, assume any reference to GPCR4 to encompass all variants.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence for GPCR4a has 591 of 940 bases (62%) identical to and 591 of 940 bases (62%) positive with *Rattus norvegicus* species taste bud Receptor clone (GENBANK-ID: U50948) (SEQ ID NO:44) (Table 4E). The residues that differs between GPCR4a and GPCR4b are highlighted in black and marked with the (o) symbol.

Table 4E. BLASTN of GPCR4a against rat TB567 gene

>gb:GENBANK-ID:RNU50948|acc:U50948 *Rattus norvegicus* taste bud receptor protein TB 567 (TB 567) gene, complete cds - *Rattus norvegicus*, 1299 bp. (SEQ ID NO:44)
Score = 1221 (183.2 bits), Expect = 3.3e-49, P = 3.3e-49
Identities = 591/940 (62%), Positives = 591/940 (62%), Strand = Plus / Plus

```
Query:    25 AATTTCACCGATGTGACAGAGTTCATTCTTTTGGGGCTAACGAG-CCGTCGAGAATGGCA 83
             |||  |||||| || || || |||  |||| ||||  |   ||| ||  | | |||
Sbjct:    46 AATGCCACCGAAGTCACTGACTTCTATCTTCTGGGATTTG-GAGTCCAGCAA-AATACTC 103

Query:    84 AGT-TCTCTTCTTCATCATCTTTCTTGTGGTCTACATCATCA-CCATGGTGGGCAATATC 141
             ||| | || |||||||  | ||| ||||| |||| ||| || ||||||||||||| |
Sbjct:   104 AGTGTGTCCTCTTCATTGTATTTTTTGTGATCTATGTCA-CATCCATGGTGGGCAACACT 162

Query:   142 GGCATGATGGTGTTAATCAAGG-TCAGTCCTCAGCTTAACAACCCCATGTACTTTTTCCT 200
             || |||||  |  | |||||  | | |||  | ||| | |  ||||||||||| ||| |
Sbjct:   163 GGGATGATCCTCCTCATCAACACTAACTCCAGA-CTTCAGACTCCCATGTACTTCTTCTT 221

Query:   201 -CAGTCACTTGTCATTTGTTGATGTGTGGTTTTCTTCCAATGTCACCCCTAAAATGTTGG 259
              ||   || || | ||||| ||| | || |   | ||      |||| || || ||| ||
Sbjct:   222 ACAAA-ACCTGGCTTTTGTGGATATCTGTTACACGTCTGCCATCACTCCCAAGATGCTGC 280

Query:   260 AAAACCTGTTTTCAGATAAAAAAACAATTACTTATGCTGGTTGTTTAGTACAGTGTTTCT 319
             | | | |  |   |||  |      | ||  | ||  | || ||| || |||| |  ||
Sbjct:   281 AGAGCTTCATGGTAGAAGACTGTTCCATATCATACACAGGATGTGTAATACAATTGTTGG 340

Query:   320 TCTTCATTGCTCTTGTCCATGTGGAAATTTTTAT-T-CTTGCTGCGATGGCCTTTGATAG 377
```

```
Sbjct:   341 TAT--ATGCCACATTTGCAACCAGTGACTGTTACCTACTCGCTGTTATGGCAGTGGACCG 398

Query:   378 ATACATGGCAATTGGGAATCCTCTGCTTTATGGCAGTAAA-ATGTCAAGGGTTGTCTGTA 436
Sbjct:   399 GTATGTGGCAATCTGTAAGCCCCTCCGGTACCCGA-TAATCATGTCTCGACAGGTCTGCT 457

Query:   437 TTCGACTGATTACTTTCCCTTACATTTATGGTTTTCTGACGAGTC-TG-GCAGCA-ACAT 493
Sbjct:   458 TACTGTTGGTCGCTCTTTCTTATCTC-ATGGGATCAATAA-ATTCCTCTGTA-CACACAG 514

Query:   494 TATGGACTTACGGCTTGTACTTCTGTGGAAAA--ATTGAGATCAACCATTTCTACTGTGC 551
Sbjct:   515 GATTTACATTCTCATTGT-CTTAT-TGTAACTCCAAAAATATCAATCACTTTTTCTGTGA 572

Query:   552 AGATCCACCTCTCATCAAAATGGCCTGTGCCGGGACCTTTGTAAAAGAAT-ATACAA-TG 609
Sbjct:   573 TGTTGTCCCAATCATCAGTCTTTCATGCTC-GA-ACACTGATATTAATATCATGCTACTT 630

Query:   610 ATCATACTTGCCGGCATTAACTTCACATATTCCCTGACTGTAATTATCATCTCTTACTTA 669
Sbjct:   631 ATTGTTTTTGTTGGATTTAACCTGACATTCACTGTGTTGGTCATTATCTTCTCTTACATA 690

Query:   670 TTCATCCTCATTGCCATTCTGCGAATGCGCTCAGCAGAAGGAAGGCAGAAGGCCTTTTCC 729
Sbjct:   691 TACATCATGGCCGCCATCCTAAAGATGTCCTCTACTGCAGGGAGGAAGAAAACCTTCTCC 750

Query:   730 ACATGTGGGTCCCATCTGACAGCTGTCATTATATTCTATGGTACTCTGATCTTC-ATGTA 788
Sbjct:   751 ACGTGTGCCTCCCACCTGACAGCAGTCACCATTTTCTATGGAACCCTT-TCTTATATGTA 809

Query:   789 TCT-CAGACGTCCCACAGAGGAGTCTGTGGAGCAGGGGAAGATGGTGGCTGTGTTCTATA 847
Sbjct:   810 CTTACAGCC-TCATTCGGACAATTCTGAGGAGAATATGAAAGTGGCCTCTGTGTTTTATG 868

Query:   848 CCACAGTGATCCCCATGTTGAATCCCATGATCTACAGTCTGAGGAACAAGGATGTGAAA- 906
Sbjct:   869 GCATTGTGATTCCCATGCTGAACCCTCTCATCTACAGCTTGAGAAACAAGGAAGTCAAAG 928

Query:   907 AAGGCCATGATGAAAGTGATCAGCAGATCATGTTAAACAAAATAAAATCAAAT--TTGAT 964
Sbjct:   929 AAGGTTTTAAAGCAA-TGAGCAGAAGGT--TCTTAAG---AATGAAATCAAATCCTTGAT 982
```

BLASTN alignments also found homology between two fragments of GPCR4 and *Mus musculus* odorant receptor M72 (GENBANK-ID:AF247656) shown if Table 4F. M72 residues 821-890 (SEQ ID NO:45) and residues 160-201 (SEQ ID NO:46), are aligned with GPCR4 in Table 4F.

Table 4F. BLASTN of GPCR4a against M72

```
>gb|AF247656.1|AF247656 Mus musculus odorant receptor M72 (M72) gene, complete cds
          Length = 930
 Score = 83.8 bits (42), Expect = 2e-13
 Identities = 63/70 (90%), Strand = Plus / Plus

Query: 836 ctgtgttctataccacagtgatccccatgttgaatcccatgatctacagtctgaggaaca 895
           ||||||||||| ||||||||||||||||||||| || ||| |||||||||| ||||| ||||
Sbjct: 821 ctgtgttctacaccacagtgatccccatgttcaacccccctgatctacagcctgagaaaca 880

Query: 896 aggatgtgaa 905
           |||| |||||
Sbjct: 881 aggaggtgaa 890  (SEQ ID NO:45)

 Score = 44.1 bits (22), Expect = 0.13
```

```
Identities = 37/42 (88%), Strand = Plus / Plus

Query: 172 cagcttaacaagcccatgtacttttttcctcagtcacttgtca 213
           |||||| ||| ||||||||||||| ||||||||| || |||||
Sbjct: 160 cagcttcacacccccatgtacttcttcctcagtaacctgtca 201  (SEQ ID NO:46)
```

BLASTN alignments found homology between fragments of GPCR4 and *Mus musculus* odorant receptor K42 (GENBANK-ID: AF282291)(SEQ ID NO:47) shown if Table 4G.

Table 4G.-BLASTN of GPCR4b against OR K42

```
>gb|AF282291.1|AF282291 Mus musculus odorant receptor K42 gene, complete cds
 (SEQ ID NO:47) Length = 927
 Score = 77.8 bits (39), Expect = 1e-11
 Identities = 60/67 (89%);  Strand = Plus / Plus

Query: 836 ctgtgttctataccacagtgatccccatgttgaatcccatgatctacagtctgaggaaca 895
           |||||||||| |||||||||||||| ||| | |||||| | || ||||||||||||||||
Sbjct: 815 ctgtgttctacaccacagtgatcccaatgctaaatcccctcatatacagtctgaggaaca 874

Query: 896 aggatgt 902
           |||||||
Sbjct: 875 aggatgt 881
```

BLASTN alignments found homology between GPCR4 and *Mus musculus* odorant receptor K40 (GENBANK-ID:AF282289) (SEQ ID NO:48) shown in Table 4G.

Table 4H.-BLASTN of GPCR4 against OR K40

```
>gb|AF282289.1|AF282289 Mus musculus odorant receptor K40 gene, complete cds
 (SEQ ID NO:48) Length = 927
 Score = 75.8 bits (38), Expect = 4e-11
 Identities = 62/70 (88%); Strand = Plus / Plus

Query: 836 ctgtgttctataccacagtgatccccatgttgaatcccatgatctacagtctgaggaaca 895
           |||||||||||||||||||||||||||| |||| ||  | || |||||| |||||||||
Sbjct: 824 ctgtgttctataccacagtgatccccatgctgaacccattaatatacagtttgaggaaca 883

Query: 896 aggatgtgaa 905
           | ||||||||
Sbjct: 884 aagatgtgaa 893
```

The full GPCR4a amino acid sequence has 155 of 304 amino acid residues (50 %) identical to, and 215 of 304 residues (70%) positive with, the 314 amino acid residue proteins from *Pan troglodytes* OR93CH (ptnr: SPTREMBL-ACC: O77756) (SEQ ID NO:41) (Table 4I). The residue that differs between GPCR4a and GPCR4b are highlighted in black and marked with the (o) symbol.

Table 4I. BLASTX of GPCR4a against OR93CH

```
>ptnr:SPTREMBL-ACC:O77756 OLFACTORY RECEPTOR OR93CH - Pan troglodytes (Chimpanzee),
314 aa. (SEQ ID NO:41)
Score = 806 (283.7 bits), Expect = 2.0e-79, P = 2.0e-79
Identities = 155/304 (50%), Positives = 215/304 (70%), Frame = +1
                                    o                   o
Query:    25 NFTDVTEFILLGLTSRREWQVLFFIIFLVV-YIITMVGNIGMMVLIKVSPQLNNPMYFFL 201
             |+| |||||  ||    + ||  |++||   |+| + ||+|||++|||++  +|+ ||||||
Sbjct:     5 NYTKVTEFIFTGLNYNPQLQVFLFLLFLTTFYVINVTGNLGMIVLIRIDSRLHTPMYFFL 64
```

```
                                        o
Query:  202 SHLSFVDVWFSSNVTPKMLENLFSDKKTITYAGCLVQCFFFIALVHVEIFILAAMAFDRY 381
            |||||||+ ||| |+|||| + |   +| |++ || +| +||    |   |+||+||+|||
Sbjct:   65 SHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMAYDRY 124

Query:  382 MAIGNPLLYGSKMSRVVCIRLITFPYIYGFLTSLAATLWTYGLYFCGKIEINHFYCADPP 561
            +|| |||||     ||+ +||+|+  ||+ | + ++  |    + | |||    ||||+|    |
Sbjct:  125 VAICNPLLYSVAMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGPNVINHFFCDMSP 184
                             o
Query:  562 LIKMACAGTFVKEYTMIILAGINFTYSLTVIIISYLFILIAILRMRSAEGRQKAFSTCGS 741
            |+ + || | + +  + |+||     +|    |+|||++||+||||+|||+|| | |||| |
Sbjct:  185 LLSLVCADTRLNKLAVFIVAGAAGVFSGLTILISYIYILMAILRIRSADGRCKTFSTCSS 244

Query:  742 HLTAVIIFYGTLIFMYLRRPTEESVEQGKMVAVFYTTVIPMLNPMIYSLRNKDVKKAMMK 921
            ||||| | |||| |+|+|      |++  |+|+|||| ||||||||+|||||||||+|| |+ +
Sbjct:  245 HLTAVFILYGTLFFIYVRPSASFSLDLNKLVSVFYTAVIPMLNPLIYSLRNKEVKDAIHR 304

Query:  922 VISR 933
             +++
Sbjct:  305 TVTQ 308
```

The GPCR4a amino acid sequence has 139 of 303 amino acid residues (45%) identical to, and 192 of 303 residues (62%) positive with the 303 amino acid OR93Gib protein from *Hylobates lar* (GENBANK-ID:AAC63971.1) (SEQ ID NO:43) (Table 4J). The residue that differs between GPCR4a and GPCR4b are highlighted in black and marked with the (o) symbol.

Table 4J–BLASTX of GPCR4a against OR93Gib

```
>gb|AAC63971.1| (AF045580) olfactory receptor OR93Gib [Hylobates lar]
(SEQ ID NO:43) Length = 313
 Score =  272 bits (695), Expect = 4e-72
 Identities = 155/303 (51%), Positives = 208/303 (69%)
                                o                         o
Query: 3   NFTDVTEFILLGLTSRREWQVLFFIIFLVVYIITMVGNIGMMVLIKVSPQLNNPMYFFLS 62
            |+| |||||  ||      + !    |  ||   |  |++ ||  ||+|||++   +|+ ||||||
Sbjct: 5   NYTKVTEFIFTGLNYNPQLQVFLFLLFLTFYVISVTGNFGMIVLIRMDSRLHTPMYFFLS 64
                                 o
Query: 63  HLSFVDVWFSSNVTPKMLENLFSDKKTITYAGCLVQCFFFIALVHVEIFILAAMAFDRYM 122
           ||||||+ ||| |+|||| + |   +| |++ || +| +||    |   |+||+||+|||+
Sbjct: 65  HLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMAYDRYV 124

Query: 123 AIGNPLLYGSKMSRVVCIRLITFPYIYGFLTSLAATLWTYGLYFCGKIEINHFYCADPPL 182
           || |||||    ||+ +||+|+  ||+ | + ++  |    + | |||    ||||+|    ||
Sbjct: 125 AICNPLLYSVFMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGLNVINHFFCDMSPL 184
                            o
Query: 183 IKMACAGTFVKEYTMIILAGINFTYSLTVIIISYLFILIAILRMRSAEGRQKAFSTCGSH 242
           + + || | + +  + |+||     +|    | |||   || ||||+|||+|| | |||| ||
Sbjct: 185 LSLVCADTRLNKLAVFIMAGAVGVFSGLTILISYIYILMAILRIRSADGRCKTFSTCSSH 244

Query: 243 LTAVIIFYGTLIFMYLRRPTEESVEQGKMVAVFYTTVIPMLNPMIYSLRNKDVKKAMMKV 302
           |||| | |||| |+|+|        ++  |+|+|||| ||||||||+|||||||||+|| |+ +
Sbjct: 245 LTAVFILYGTLFFIYVRPSASFPLDLNKVVSVFYTAVIPMLNPLIYSLRNKEVKDAIHRT 304

Query: 303 ISR 305
           +++
Sbjct: 305 VTQ 307
```

The GPCR4a protein has 137 of 304 amino acid residues (45%) identical to, and 191 of 304 residues (62%) positive with, the 308 amino acid odorant receptor K42 from *Mus musculus* (GENBANK-ID:AAG39876.1) (SEQ ID NO:49) (Table 4K). The residue that

differs between GPCR4a and GPCR4b are highlighted in black and marked with the (o) symbol.

Table 4K. BLASTX of GPCR4a against K42

```
>gb|AAG39876.1| (AF282291) odorant receptor K42 [Mus musculus] (SEQ ID NO:49), 308
aa
Statistics for GPCR4a:  Score =  271 bits (694), Expect = 6e-72
                        Identities = 149/304 (49%), Positives = 203/304 (67%)
Statistics for GPCR4b:  Score =  273 bits (699), Expect = 2e-72
                        Identities = 138/304 (45%), Positives = 192/304 (62%)

Query: 2   LNFTDVTEFILLGLTSRREWQVLFFIIFLVVYIITMVGNIGMMVLIKVSPQLNNPMYFFL 61
           +| + ||+||| ||| | | |   |  |     ||+|||+||++|| +| ||++|||+||
Sbjct: 1   MNHSSVTDFILEGLTKRPELQLPLFLLFLGIHVITVVGNLGMILLINISSQLHSPMYYFL 60

Query: 62  SHLSFVDVWFSSNVTPKMLENLFSDKKTITYAGCLVQCFFFIALVHVEIFILAAMAFDRY 121
           |||||+|+ +|| +||||| |    | ||++  |+ | +||+ |   | ++| |||+|||
Sbjct: 61  SHLSFIDLCYSSVITPKMLVNFVCAKNTISFKECMTQLYFFLLLAISEGYLLTAMAYDRY 120

Query: 122 MAIGNPLLYGSKMSRVVCIRLITFPYIYGFLTSLAATLWTYGLYFCGKIEINHFYCADPP 181
           +|| +|||| + ||  ||  ++   |  ||    + |     | |||   | |++|   |
Sbjct: 121 VAICSPLLYNTVMSHKVCSIMMAVVYSLGFFGATVHTTRMTMLSFCGSHIIRHYFCDILP 180

Query: 182 LIKMACAGTFVKEYTMIILAGINFTYSLTVIIISYLFILIAILRMRSAEGRQKAFSTCGS 241
           |+ ++|+ | + |  + |+ |+|        |||| |||  |||+|| ||| ||| || |
Sbjct: 181 LLTLSCSSTHINEVLLFIIGGVNTLAPTLAVIISYAFILTSILRIRSNEGRSKAFGTCSS 240

Query: 242 HLTAVIIFYGTLIFMYLRRPTEESVEQGKMVAVFYTTVIPMLNPMIYSLRNKDVKKAMMK 301
           |+ || ||+|++ ||| + |+  ++|| |+ +||||||||||||+|||||||||| |+ |
Sbjct: 241 HIMAVGIFFGSITFMYFKPPSSNNMEQEKVSSVFYTTVIPMLNPLIYSLRNKDVKTALKK 300

Query: 302 VISR 305
           ++ |
Sbjct: 301 MVGR 304
```

The GPCR4b protein has 141 of 301 amino acid residues (46%) identical to, and 189 of 301 residues (61%) positive with, the 314 amino acid OR 5I1 from *Mus musculus* (GENBANK-ID:AAG39876.1) (SEQ ID NO:50) (Table 4L). The residue that differs between GPCR4a and GPCR4b are highlighted in black and marked with the (o) symbol.

Table 4L. BLASTX of GPCR4b against OR 5I1

```
ref|NP_006628.1| olfactory receptor, family 5, subfamily I, member 1 [Homo sapiens]
sp|Q13606|O5I1_HUMAN OLFACTORY RECEPTOR 5I1 (OLFACTORY RECEPTOR-LIKE PROTEIN OLF1)
gb|AAB01214.1| (U56420) HsOLF1 [Homo sapiens]  (SEQ ID NO:50); Length = 314
 Score =  274 bits (700), Expect = 1e-72
 Identities = 141/301 (46%), Positives = 189/301 (61%)
                                    o                o
Query: 3   NFTDVTEFILLGLTSRREWQVLFFIVFLVVYIITMVGNIGMMLLIKVSPQLNNPMYFFLS 62
           |+| ||||||||  +| | ||  |  ||  | | ++||||+||||++ | |  |||||||
Sbjct: 7   NYTLVTEFILLGFPTRPELQIVLFLMFLTLYAIILIGNIGLMLLIRIDPHLQTPMYFFLS 66
                                o
Query: 63  HLSFVDVWFSSNVTPKMLENLLSDKKTITYAGCLVQCFFFIALVHVEIFILAAMAFDRYM 122
           +|||||+ + |++ |||| | ||+ |+|+| || +| +||      | |||||||+|||+
Sbjct: 67  NLSFVDLCYFSDIVPKMLVNFLSENKSISYYGCALQFYFFCTFADTESFILAAMAYDRYV 126

Query: 123 AIGNPLLYGSKMSRVVCIRLITFPYIYGFLTSLAATLWTYGLYFCGKIEINHFYCADPPL 182
           || |||||   ||| +|+|||   |+ | ++||  | + + | +| |  ||||+|  |||
Sbjct: 127 AICNPLLYTVVMSRGICMRLIVLSYLGGNMSSLVHTSFAFILKYCDKNVINHFFCDLPPL 186
                          o
Query: 183 IKMACAGTFVKEYTMLILAGINFTYSLTVIIISYLFILIAILRMRSAEGRQKAFSTCGSH 242
           +|++|  | + |+ +             +||||| |||   |++||  ||+| |||| ||
```

```
Sbjct: 187 LKLSCTDTTINEWLLSTYGSSVEIICFIIIIISYFFILLSVLKIRSFSGRKKTFSTCASH 246

Query: 243 LTAVIIFYGTLIFMYLRRPTEESVEQGKMVAVFYTTVIPMLNPMIYSLRNKDVKKAMMKV 302
           ||+| |+ |||+|+|+| |         |       |+++||||  ||+|||+|||||||||| |   ||
Sbjct: 247 LTSVTIYQGTLLFIYSRPSYLYSPNTDKIISVFYTIFIPVLNPLIYSLRNKDVKDAAEKV 306

Query: 303 I 303
           +
Sbjct: 307 L 307
```

The full amino acid sequence of the GPCR4b protein has 140 of 303 amino acid residues (46%) identical to, and 193 of 303 residues (63%) positive with, the 312 amino acid OR4 protein from *Mus musculus* (GENBANK-ID:AAG39876.1) (SEQ ID NO:34) (Table 4J). The residue that differs between GPCR4a and GPCR4b are highlighted in black and marked with the (o) symbol.

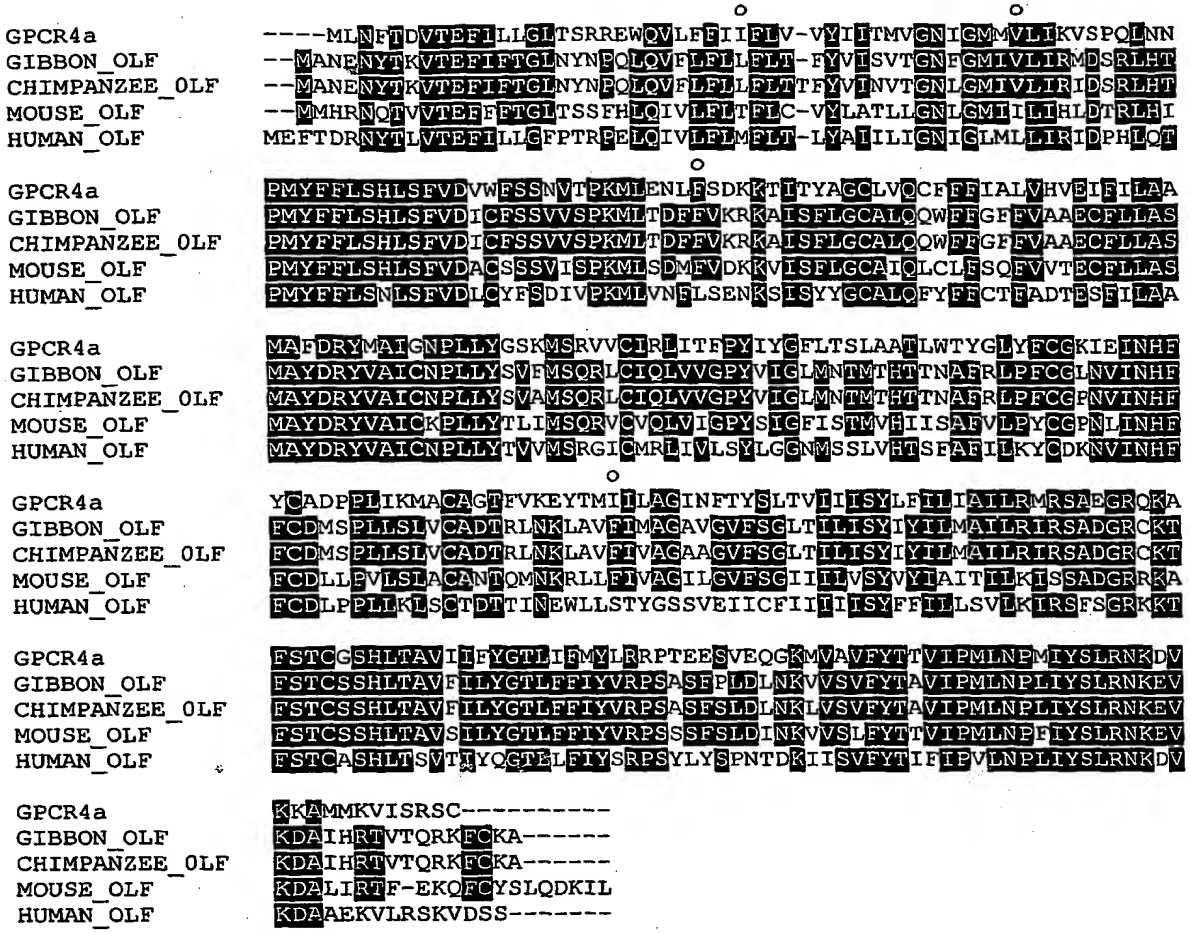
Table 4M.-BLASTX of GPCR4b and OR 4

```
emb|CAA64370.1| (X94744) olfactory receptor 4 [Gallus gallus]; (SEQ ID NO:34)
  Length = 312; Score = 271 bits (693), Expect = 7e-72
  Identities = 140/303 (46%), Positives = 193/303 (63%)

                                          o                        o
Query: 3   NFTDVTEFILLGLTSRREWQVLFFIVFLVVYIITMVGNIGMMLLIKVSPQLNNPMYFFLS 62
           | |   +||||+||+    + +   | |||  | ||  ||+||++||+   |+|+  ||||||
Sbjct: 5   NHTLASEFILVGLSDHPKMKAALFVVFLLIYVITFQGNLGIIILIQGDPRLHTSMYFFLS 64
                                 o
Query: 63  HLSFVDVWFSSNVTPKMLENLLSDKKTITYAGCLVQCFFFIALVHVEIFILAAMAFDRYM 122
            || ||+ ||| + |+ | | ||+++||++ ||  | ||+|  |  | |+|| ||+|||+
Sbjct: 65  SLSVVDICFSSVIAPRTLVNFLSERRTISFTGCTGQTFFYIVFVTTECFLLAVMAYDRYV 124

Query: 123 AIGNPLLYGSKMSRVVCIRLITFPYIYGFLTSLAATLWTYGLYFCGKIEINHFYCADPPL 182
           || ||||| + |+| |++|+  || | | ++ |+ |  |||||||||||||||||||||
Sbjct: 125 AIGNPLLYSTIMTRRQCMQLVVGSYIGGILNAIIQTTFIIRLPFCGSNIINHFFCDVPPL 184
                          o
Query: 183 IKMACAGTFVKEYTMLILAGINFTYSLTVIIISYLFILIAILRMRSAEGRQKAFSTCGSH 242
           + ++ | |++ | + ||||  ++| | || || ||||+|||||||||| ||| ||
Sbjct: 185 LALSLASTYISEMILFSLAGIIELSTVTSILVSYIFISCAILRIRSAEGRQKALSTCASH 244

Query: 243 LTAVIIFYGTLIFMYLRRPTEESVEQGKMVAVFYTTVIPMLNPMIYSLRNKDVKKAMMKV 302
           |||| + ||| || ||| + |+ |+|+|||| |||||||+||||||++|| |+ +|
Sbjct: 245 LTAVTLLYGTTIFTYLRPSSSYSLNTDKVVSVFYTVVIPMLNPLIYSLRNQEVKGALSRV 304

Query: 303 ISR 305
           + |
Sbjct: 305 VER 307
```

A multiple sequence alignment is given in Table 4K, with GPCR4a being shown on line 1, in a ClustalW analysis comparing GPCR4a with related protein sequences.

Table 4N.-Information for the ClustalW proteins:

1. Novel Human_OLF, GPCR4a, SEQ ID NO:10
2. *Hylobates lar* (Common Gibbon) OLF, SPTREMBL-Acc # O77758, SEQ ID NO:43
3. *Pan troglodytes* (Chimpanzee) OLF, SPTREMBL-Acc # O77756, SEQ ID NO:41
4. *Mus musculus* OLF, GENBANK-Acc # AAF20365, SEQ ID NO:42
5. *Homo sapiens* OLF, SWISSPROT-Acc # Q13606, SEQ ID NO:33



DOMAIN results for GPCR4a were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 4L with the statistics and domain description. Residues 1-163 (SEQ ID NO:29) and residues 313-377 (SEQ ID NO:37) of 7tm_1 are aligned with GPCR4 in Table 4O. The residue that differs between GPCR4a and GPCR4b are highlighted in black and marked with the (o) symbol.

Table 4O. DOMAIN results for GPCR4a.

```
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family)
(SEQ ID NO: 29)  Length = 377
  Statistics for GPCR4a:   Score = 89.7 bits (221), Expect = 2e-19
  Statistics for GPCR4b:   Score = 90.5 bits (223), Expect = 1e-19

Query: 39   GNIGMMVLIKVSPQLNNPMYFFLSHLSFVDVWFSSNVTPKMLENLFSDKKTITYAGCLVQ  98
Sbjct: 1    GNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF  60
             **: : : :     *     : :  *:  *:  :: * * ::      :   :   * :

Query: 99   CFFFIALVHVEIFILAAMAFDRYMAIGNPLLYGSKMSRV-VCIRLITFPYIYGFLTSLAA  157
Sbjct: 61   VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVWVLSFTISCPM  120
                : :    *  * *:: *** *:  *:** :: *       :*   ::  *  *

Query: 158  TLWTYGLYFCGKIEINHFYCADPPLIKMACAGTFVKEYTMIILAGINFTYSLTV  211
Sbjct: 121  LFGLNNTDQNE---CIIAN---PAFVVYSSIVSFYVPFIVTL-----LVYIKIY  163
                              *  :  :   :*   : : :       *
```

```
Sbjct: 7 transmembrane receptor (rhodopsin family) fragment (SEQ ID NO: 37)
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family) 377 aa
  Statistics for GPCR4a:   Score = 40.4 bits (93), Expect = 1e-04
  Statistics for GPCR4b:   Score = 40.4 bits (93), Expect = 1e-04

Query: 225  R  SAEGRQKAFSTCGSHLTAVIIFYGTLIFMYLRRPTEESVEQG-KMVAVFYTTVIPML  283
Sbjct: 313  K  QQKEKKATQMLAIVLGVFIICWLPFFITHILNIHCDCNIPPVLYSAFTWLGYVNSAV  372
             ::   : ::            :* :    *   *    : ::         :   *   :

Query: 284  NP  Y  288
Sbjct: 373  NP  Y  377
             **:**
```

The nucleic acids and proteins of GPCR4 are useful in potential therapeutic applications implicated in various in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor-like protein may be useful in gene therapy, and the olfactory receptor-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Ostoeodystrophy. Other GPCR-related diseases and disorders are contemplated.

The novel GPCR4 nucleic acid and protein, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. This novel protein also has immense value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders

GPCR5

GPCR5 is an Olfactory Receptor ("OR")-like protein, wherein three alternative novel GPCR5 nucleic acids and encoded polypeptides are disclosed.

The novel GPCR5a nucleic acid of 980 nucleotides (also referred to as AP001112_C) is shown in Table 5A. An ORF begins with an ATG initiation codon at nucleotides 26-28 and ends with a TGA codon at nucleotides 941-43. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 5A, and the start and stop codons are in bold letters.

Table 5A. GPCR5a Nucleotide Sequence (SEQ ID NO:13)

AGCTTGAAGAGCAAACTGTCAGGAAATGTCCAACACAAATGGCAGTGCAATCACAGAATTCATTTTACTTGGGCTCACAGATTG
CCCGGAACTCCAGTCTCTGCTTTTTGTGCTGTTTCTGGTTGTTTACCTCGTCACCCTGCTAGGCAACCTGGGCATGATAATGTT
AATGAGACTGGACTCTCGCCTTCACACGCCCATGTACTTCTTCCTCACTAACTTAGCCTTTGTGGATTTGTGCTATACATCAAA
TGCAACCCCGCAGATGTCGACTAATATCGTATCTGAGAAGACCATTTCCTTTGCTGGTTGCTTTACACAGTGCTACATTTTCAT
TGCCCTTCTACTCACTGAGTTTTACATGCTGGCAGCAATGGCCTATGACCGCTATGTGGCCATATATGACCCTCTGCGCTACAG
TGTGAAAACGTCCAGGAGAGTTTGCATCTGCTTGGCCACATTTCCCTATGTCTATGGCTTCTCAGATGGACTCTTCCAGGCCAT
CCTGACCTTCCGCCTGACCTTCTGTAGATCCAATGTCATCAACCACTTCTACTGTGCTGACCCGCCGCTCATTAAGCTTTCTTG
TTCTGATACTTATGTCAAAGAGCATGCCATGTTCATATCTGCTGGCTTCAACCTCTCCAGCTCCCTCACCATCGTCTTGGTGTC
CTATGCCTTCATTCTTGCTGCCATCCTCCGGATCAAATCAGCAGAGGGAAGGCACAAGGCATTCTCCACCTGTGGTTCCCATAT
GATGGCTGTCACCCTGTTTTATGGGACTCTCTTTTGCATGTATATAAGACCACCAACAGATAAGACTGTTGAGGAATCTAAAAT
AATAGCTGTCTTTTACACCTTTGTGAGTCCGGTACTTAATCCATTGATCTACAGTCTGAGGAATAAAGATGTGAAGCAGGCCTT
GAAGAATGTCCTGAGATGAAATATTGTCATGACCATGGTGATGCCTTTGTTTCCTA

The GPCR5a protein encoded by SEQ ID NO:13 has 305 amino acid residues, and is presented using the one-letter code in Table 5B. The SignalP, Psort and/or Hydropathy profile for GPCR5a predict that GPCR5a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The signalP shows a signal sequence is coded for in the first 44 amino acids, *i.e.*, with a cleavage site at the dash in the sequence NLG-MIM, between amino acids 44 and 45. This is typical of this type of membrane protein.

Table 5B. Encoded GPCR5a protein sequence (SEQ ID NO:14).

MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMYFFLTNLAFVDLCYTSNATPQMSTN
IVSEKTISFAGCFTQCYIFIALLLTEFYMLAAMAYDRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFC
RSNVINHFYCADPPLIKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYAFILAAILRIKSAEGRHKAFSTCGSHMMAVTLFYG
TLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQALKNVLR

The target GPCR5a sequence, above, was subjected an the exon linking process to confirm the sequence, as reported for GPCR2 and GPCR4, above. The resulting GPCR5b sequence (also referred to herein as AC0170103B1) is reported below in Table 5C .

Table 5C. GPCR5 Nucleotide Sequence (SEQ ID NO:15)

AGCTTGAAGAGCAAACTGTCAGGAAATGTCCAACACAAATGGCAGTGCAATCACAGAATTCATTTTACTTGGGCTCACAGATTG
CCCGGAACTCCAGTCTCTGCTTTTTGTGCTGTTTCTGGTTGTTTACCTCGTCACCCTGCTAGGCAACCTGGGCATGATAATGTT
AATGAGACTGGACTCTCGCCTTCACACGCCCATGTACTTCTTCCTCACTAACTTAGCCTTTGTGGATTTGTGCTATACATCAAA
TGCAACCCCGCAGATGTCGACTAATATCGTATCTGAGAAGACCATTTCCTTTGCTGGTTGCTTTACACAGTGCTACATTTTCAT
TGCCCTTCTACTCACTGAGTTTTACATGCTGGCAGCAATGGCCTATGACCGCTATGTGGCCATATATGACCCTCTGCGCTACAG
TGTGAAAACGTCCAGGAGAGTTTGCATCTGCTTGGCCACATTTCCCTATGTCTATGGCTTCTCAGATGGACTCTTCCAGGCCAT
CCTGACCTTCCGCCTGACCTTCTGTAGATCCAGTGTCATCAACCACTTCTACTGTGCTGACCCGCCGCTCATTAAGCTTTCTTG
TTCTGATACTTATGTCAAAGAGCATGCCATGTTCATATCTGCTGGCTTCAACCTCTCCAGCTCCCTCACCATCGTCTTGGTGTC
CTATGCCTTCATTCTTGCTGCCATCCTCCGGATCAAATCAGCAGAGGGAAGGCACAAGGCATTCTCCACCTGTGGTTCCCATAT
GATGGCTGTCACCCTGTTTTATGGGACTCTCTTTTGCATGTATATAAGACCACCAACAGATAAGACTGTTGAGGAATCTAAAAT
AATAGCTGTCTTTTACACCTTTGTGAGTCCGGTACTTAATCCATTGATCTACAGTCTGAGGAATAAAGATGTGAAGCAGGCCTT
GAAGAATGTCCTGAGATGAAATATTGTCATGACCATGGTGATGCCTTTGTTTCCTA

The GPCR5b protein encoded by SEQ ID NO:15 has 305 amino acid residues and is presented using the one-letter code in Table 5D. The SignalP, Psort and/or Hydropathy profiles for GPCR5b are the same as for GPCR5a.

Table 5D. Encoded GPCR5 protein (SEQ ID NO:16).

MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMYFFLTNLAFVDLCYTSNATPQMSTN
IVSEKTISFAGCFTQCYIFIALLLTEFYMLAAMAYDRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFC
RSSVINHFYCADPPLIKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYAFILAAILRIKSAEGRHKAFSTCGSHMMAVTLFYG
TLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQALKNVLR

In an alternative embodiment, a novel GPCR5c nucleic acid of 1006 nucleotides (also referred to herein as CG50173-01) is shown in Table 5E. An ORF was identified beginning with an ATG initiation codon at nucleotides 83-85 and ending with a TGA codon at nucleotides 998-1000. Putative untranslated regions, if any, are found upstream from the initiation codon and downstream from the termination codon.

Table 5E. GPCR5c Nucleotide Sequence (SEQ ID NO:17)

```
AATTCAAATGAACATTAACATGGTATGTGCATTTGTTTATATTGGCTTTATTTCCATAGCTTGAAGAGCAAACTGTCAGGAAAT
GCCCAACACAAATGGCAGTGCAATCACAGAATTCATTTTACTTGGGCTCACAGATTGCCCGGAACTCCAGTCTCTGCTTTTTGT
GCTGTTTCTGGTTGTTTACCTCGTCACCCTGCTAGGCAACCTGGGCATGATAATGTTAATGAGACTGGACTCTCGCCTTCACAC
GCCCATGTACTTCTTCCTCACTAACTTAGCCTTTGTGGATTTGTGCTATACATCAAATGCAACCCCGCAGATGTCGACTAATAT
CGTATCTGAGAAGACCATTTCCTTTGCTGGTTGCTTTACACAGTGCTACATTTTCATTGCCCTTCTACTCACTGAGTTTTACAT
GCTGGCAGCAATGGCCTATGACCGCTATGTGGCCATATATGACCCTCTGCGCTACAGTGTGAAAACGTCCAGGAGAGTTTGCAT
CTGCTTGGCCACATTTCCCTATGTCTATGGCTTCTCAGATGGACTCTTCCAGGCCATCCTGACCTTCCGCCTGACCTTCTGTAG
ATCCAATGTCATCAACCACTTCTACTGTGCTGACCCGCCGCTCATTAAGCTTTCTTGTTCTGATACTTATGTCAAAGAGCATGC
CATGTTCATATCTGCTGGCTTCAACCTCTCCAGCTCCCTCACCATCGTCTTGGTGTCCTATGCCTTCATTCTTGCTGCCATCCT
CCGGATCAAATCAGTAGAGGGAAGGCACAAGGCATTCTCCACCTGTGGTTCCCATATGATGGCTGTCACCCTGTTTTATGGGAC
TCTCTTTTGCATGTATATAAGACCACCAACAGATAAGACTGTTGAGGAATCTAAAATAATAGCTGTCTTTTACACCTTTGTGAG
TCCGGTACTTAATCCATTGATCTACAGTCTGAGGAATAAAGATGTGAAGCAGGGCTTGAAGAATGTCCTGAGATGAAATATT
```

The GPCR5c protein encoded by SEQ ID NO:17 has 305 amino acid residues and is presented using the one-letter code in Table 5F. The SignalP, Psort and/or Hydropathy profiles for GPCR5c are the same as for GPCR5a and GPCR5b.

Table 5F. Encoded GPCR5c protein sequence (SEQ ID NO:18).

```
MPNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMYFFLTNLAFVDLCYTSNATPQMSTN
IVSEKTISFAGCFTQCYIFIALLLTEFYMLAAMAYDRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFC
RSNVINHFYCADPPLIKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYAFILAAILRIKSVEGRHKAFSTCGSHMMAVTLFYG
TLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQGLKNVLR
```

GPCR5 variants differ at four nucleotide residues, namely GPCR5a and GPCR5b differ from GPCR5c at T29C, C714T and C921G, while GPCR5a and GPCR5c differ from GPCR5b at A537G. GPCR5 variants differ at four amino acid residues, namely GPCR5a and GPCR5b differ from GPCR5c at S2P, A230V and A299G, while GPCR5a and GPCR5c differ from GPCR5b at N171S. All numbering is in reference to GPCR5a. Unless specifically addressing GPCR5a or GPCR5b or GPCR5c, assume any reference to GPCR5 to encompass all variants.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence GPCR5a has 633 of 959 bases (66 %) identical to and 633 of 959 bases (66%) positive with a *Gallus gallus* species Olfactory Receptor clone (GENBANK-ID: X94742) (SEQ ID NO:51) (Table 5G). The residue that differs between GPCR5a, GPCR5b and GPCR5c are highlighted in black and marked with the (o) symbol.

Table 5G. BLASTN of GPCR5a against OR 2 (SEQ ID NO:51)

```
>gb:GENBANK-ID:GGCOR2GEN|acc:X94742 G. gallus cor2 DNA for olfactory receptor 2
Gallus gallus, 996 bp.  (SEQ ID NO:51)
Statistics for GPCR5a:   Score = 1409 (211.4 bits), Expect = 1.4e-57, P = 1.4e-57
                         Identities = 633/959 (66%), Positives = 633/959 (66%),
Statistics for GPCR5c:   Score = 1379 (206.9 bits), Expect = 2.5e-56, P = 2.5e-56
                         Identities = 623/944 (65%), Positives = 623/944 (65%)
Strand = Plus / Plus

Query:     7 AAGAGCAAACTGTCA-G-GAA-ATGTCCAACACAAATGGCAGTGCAATCACAGAATTCAT 63
Sbjct:    37 AACTGCAA-CTGTGTTGTGATGATGGCCAAGGGAAATCACAGCTCCATCACTGAATTTGT 95

Query:    64 TTTACTTGGGCT-CACAGATTGCCCGGAACTCCAGTCTCTGCTTTTTGTGCTGTTTCTGG 122
Sbjct:    96 GCT-CTTGGGATTCTCTGAAAAGAGGGCCATCCAGGCTGTTCTCTTTATGG-GCTTCTTG 153

Query:   123 TTGTT-TACCTCGTCACCCTGCTAGGCAACCTGGGCATGATAATGTTAATGAGACTGGAC 181
Sbjct:   154 CTGATCTACCTGATCACTCTGCTAGGCAATGTGGGCATGATCACATTGATCAGGCTGGAC 213

Query:   182 TCTCGCCTTCACACGCCCATGTACTTCTTCCTCACTAACTTAGCCTTTGTGGATTTGTGC 241
Sbjct:   214 TCCCGGCTTCACACCCCTATGTACTTCTTCCTGAGCAGCTTGTCCTTCCTCGATATCTGC 273

Query:   242 TATACATC-A-AATGCAACCC-CGCA-GATGTC-GACTAATATC-GTATCTGAGAAGACC 295
Sbjct:   274 TATTCCTCCACAAT-CACTCCTCGAGTGCTCTCAGACC--TCCCAGCATCACAGAAAGTC 330

Query:   296 ATTTCCTTTGCTGGTTGCTTTACACAGTGCTACATTTTCATTGCCCTT-CTACTCACTGA 354
Sbjct:   331 ATTTCCCACTCTGCATGCCTGGCACAGTTTTATTTCTACGCTGTCTTTGCCAC-CACAGA 389

Query:   355 GTTTTACATGCTGGCAGCAATGGCCTATGACCGCTATGTGGCCATATATGACCCTCTGCG 414
Sbjct:   390 GTGCTATCTTTTGGCCGCAATGGCATATGACCGCTACGTGGCCATCTGCAGCCCTCTGCT 449

Query:   415 CTACAGTGTGAAA-ACGTCCAGGAGAGTTTGCAT-CTGCTTG--GCCACATT-TCCCTAT 469
Sbjct:   450 CTAT-GTCTTCTCCATGTCCAGCAGAGTTTGTGTGCTGCTGGTTGCTGGCTCATACCT-T 507

Query:   470 GTCTATGGCTTCTCAGATGG-ACTCTTCCAGGCCATCCTGAC-CTTCCGCCTGACCTTCT 527
Sbjct:   508 GTCGG-GG-TTGTGA-ATGCCACCATTC-ACACAGGGCTTGCACTGCAGC-TGTCCTTCT 562

Query:   528 GTAGATCCATGTCATCAACCACTTCTACTGTG-CTGACCCGCCGCTCATTAAGCTTTCT 586
Sbjct:   563 GTGGTCCCAACATCATCAATCACTTCTACTGTGACGGTCCC-CCGCTC-T-ACGCCATCT 619

Query:   587 TGTTCTGATACTT-ATGTCAAA-GAGCATGCCATGTTCATATCTCCT-GGCTTCAACCT- 642
Sbjct:   620 CGTGCACAGACCCCACCACCAACGAGATTGCGATATTTCT-TGTGGTTGGCTTCAACATG 678

Query:   643 CTCCAGCTCCCTCACCATCGTCTTGGTGTCCTATGCCTTCATTCTTGCT-GCCATCCTCC 701
Sbjct:   679 CTC-ATCACCAGCGTGACCATCTTCATCTCCTACACCTACATCCT-GTTCGCTGTCCTCA 736

Query:   702 GGAT-CAAATCAGCAG-AGGGAAGGCACAAGGCATTCTCCACCTGTGGTTCCCATATGAT 759
Sbjct:   737 GGATGCACA-CAGCTGCAGGCAAA-CGCAAAACCTTCTCCACGTGTGCGTCCCACCTGGC 794

Query:   760 GGCTGTCACCCTGTTTTATGGGACTCT-CTT-TTGCATGTATATAAGACC-ACCAACAGA 816
Sbjct:   795 CACCGTCACCCTATTCTATGC--CTCTGCTGGTTCCATGTACTCACGGCCCAGCTCCAGG 852

Query:   817 TAAGACT-GTTGAGGAATCTAAAATAATAGCTGTCTTTTACACCTTTGTGAGTCCGGTAC 875
Sbjct:   853 CACTCCCAGGACCTGGA-C-AAGGTGGCCTCTGTGTTCTACACCATGGTGACCCCCATGC 910
```

```
                                                                o
Query:   876 TTAATCCATTGATCTACAGTCTGAGGAATAAAGATGTGAAGCAGGCCTTGAAGAATGTCC 935
             | || ||  | ||||||| |||||||  | || || ||| | |  ||   ||| ||
Sbjct:   911 TGAACCCCCTCATCTACAGCCTGAGGAACCAGGAGGTAAAGGATGTTTTAGGGAAAGTGA 970

Query:   936 TGAGATGAAATATTGTCA-TGACCA 959
             || |  | || | ||||  |||| |
Sbjct:   971 TGGGGAGGAAGAGTGTCTCTGACAA 995
```

The full amino acid sequence of the protein of GPCR5a has 160 of 301 amino acid residues (53 %) identical to, and 215 of 301 residues (71%) positive with, the 313 amino acid OR93GIB from *Hylobates lar* (ptnr: SPTREMBL-ACC: 077758) (SEQ ID NO:43) (Table 5H). The residue that differs between GPCR5a, GPCR5b and GPCR5c are highlighted in black and marked with the (o) symbol.

Table 5H.-BLASTX of GPCR5a against OR93GIB

```
>ptnr:SPTREMBL-ACC:O77758 OLFACTORY RECEPTOR OR93GIB - Hylobates lar (Common
gibbon), 313 aa.  (SEQ ID NO:43)
Score = 803 (282.7 bits), Expect = 4.2e-79, P = 4.2e-79
Identities = 160/301 (53%), Positives = 215/301 (71%), Frame = +2
                o
Query:    26 MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMY 205
             |+| | + +|||| ||   |+|| ||+||| |++++ || |||+|+|+|||||||||
Sbjct:     1 MANENYTKVTEFIFTGLNYNPQLQVFLFLLFLTFYVISVTGNFGMIVLIRMDSRLHTPMY 60

Query:   206 FFLTNLAFVDLCYTSNATPQMSTNI-VSEKTISFAGCFTQCYIFIALLLTEFYMLAAMAY 382
             |||++|+|||+|++|  +|+| |+  |  | ||| ||  | + |   +  | ++||+|||
Sbjct:    61 FFLSHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMAY 120
                                                                o
Query:   383 DRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFCRSNVINHFYCA 562
             |||||| +|| |||  |+|+|| |   ||| |  + +      ||| ||  ||||||+|
Sbjct:   121 DRYVAICNPLLYSVFMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGLNVINHFFCD 180
                                                                o
Query:   563 DPPLIKLSCSDTYVKEHAMFISAG-FNLSSSSLTIVLVSYAFILAAILRIKSAEGRHKAFS 739
               ||+ | |+|| + + |+|| ||   + | ||| |+|| +|| |||||+||+|| | ||
Sbjct:   181 MSPLLSLVCADTRLNKLAVFIMAGAVGVFSGLTI-LISYIYILMAILRIRSAEGRCKTFS 239

Query:   740 TCGSHMMAVTLFYGTLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQ 919
             || ||+ || + ||||| +|+||     ++ +|+++|||| | |+|||||||||||+||
Sbjct:   240 TCSSHLTAVFILYGTLFFIYVRPSASFPLDLNKVVSVFYTAVIPMLNPLIYSLRNKEVKD 299
                o
Query:   920 AL 925
             |+
Sbjct:   300 AI 301
```

The GPCR5a amino acid has 154 of 306 amino acid residues (55%) identical to, and 199 of 306 residues (64%) positive with, the 309 amino acid M72 from *Mus musculus* (GENBANK-ID:AAG09870.1) (SEQ ID NO:53) (Table 5I). The residue that differs between GPCR5a, GPCR5b and GPCR5c are highlighted in black and marked with the (o) symbol.

Table 5I.-BLASTX of GPCR5a against M72

```
>gb|AAG09780.1|AF247656_1 (AF247656) odorant receptor M72 [Mus musculus]
 (SEQ ID NO:53) Length = 309
 Score =  294 bits (752), Expect = 1e-78
 Identities = 161/306 (53%), Positives = 206/306 (68%), Gaps = 2/306 (0%)
               o
Query: 1    MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMY 60
            |+  | | +|||||  |||+ ||||  |  |||  | ||  |||||| |+ |+|+||||||
```

```
Sbjct: 1   MAAENQSTVTEFILRGLTNRPELQLPLLLLFLGIYIVTMVGNLGMITLIGLNSQLHTPMY 60

Query: 61  FFLTNLAFVDLGYTSNATPQMSTNIVSEKT-ISFAGCFTQCYIFIALLLTEFYMLAAMAY 119
           |||+||+ |||||+|  ||+|  | ||++  ||+ || +| | |+  ++ | |||  |||
Sbjct: 61  FFLSNLSLVDLCYSSVITPKMLINFVSQRNLISYVGCMSQLYFFLVFVIAECYMLTVMAY 120
                                                          o
Query: 120 DRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFCRSNVINHFYCA 179
           ||||||  || |++  | +|  |  | |  |       +  |  +| +|  ++|+|++|
Sbjct: 121 DRYVAICQPLLYNIIMSPALCSLLVVFVYAMGLIGSTIETSLMLKLNYCE-DLISHYFCD 179
                                                        o
Query: 180 DPPLIKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYAFILAAILRIKSAEGRHKAFST 239
             ||+|||||  ||  | |+|  ||||+  +   ||+|||||++|||| | ||| |||||
Sbjct: 180 ILPLMKLSCSSTYDIEMAVFFLAGFNIIVTSLTVLISYAFILSSILRISSNEGRSKAFST 239
                                                                    o
Query: 240 CGSHMMAVTLFYGTLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQA 299
           | ||  || ||||+  ||++| |  ++ +  + +|||| | |+ ||||||||||+|| |
Sbjct: 240 CSSHFAAVGLFYGSTAFMYLKPSTASSLAQENVASVFYTTVIPMFNPLIYSLRNKEVKTA 299

Query: 300 LKNVLR 305
           |  ||
Sbjct: 300 LDKTLR 305
```

The GPCR5a amino acid has 148 of 301 amino acid residues (49%) identical to, and 198 of 301 residues (65%) positive with, the 308 amino acid K42 from *Mus musculus* (GENBANK-ID:AAG39876.1) (SEQ ID NO:53) (Table 5J). The residue that differs between GPCR5a, GPCR5b and GPCR5c are highlighted in black and marked with the (o) symbol.

Table 5J.-BLASTX of GPCR5a against K42

```
>gb|AAG39876.1|AF282291_1 (AF282291) odorant receptor K42 [Mus musculus]
(SEQ ID NO:53) Length = 308
 Score =  293 bits (751), Expect = 1e-78
 Identities = 153/301 (51%), Positives = 203/301 (67%), Gaps = 1/301 (0%)

Query: 5   NGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMYFFLT 64
           | |++|+||| |||  ||||  || |||        |||||+|+ + |+||+|||+||+
Sbjct: 2   NHSSVTDFILEGLTKRPELQLPLFLLFLGIHVITVVGNLGMILLINISSQLHSPMYYFLS 61

Query: 65  NLAFVDLCYTSNATPQMSTNIVSEK-TISFAGCFTQCYIFIALLLTEFYMLAAMAYDRYV 123
           +|+|+|||||+|  ||+|  | |  | ||||  | || | |+ | ++| |+| ||||||||
Sbjct: 62  HLSFIDLCYSSVITPKMLVNFVCAKNTISFKECMTQLYFFLLLAISEGYLLTAMAYDRYV 121
                                                          o
Query: 124 AIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFCRSNVINHFYCADPPL 183
           ||  || |+  | +||  +   |  ||            |+|| |++| |++|   ||
Sbjct: 122 AICSPLLYNTVMSHKVCSIMMAVVYSLGFFGATVHTTRMTMLSFCGSHIIRHYFCDILPL 181
                                                        o
Query: 184 IKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYAFILAAILRIKSEEGRHKAFSTCGSH 243
           + |||| |++ |  +||  | |  +    |++|||||| +||||+| ||| ||| || ||
Sbjct: 182 LTLSCSSTHINEVLLFIIGGVNTLAPTLAVIISYAFILTSILRIRSNEGRSKAFGTCSSH 241
                                                                  o
Query: 244 MMAVTLFYGTLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQALKNV 303
           +||| +|+|++  || +||+   +|+ |+ +|||| | |+|||||||||||| ||| +
Sbjct: 242 IMAVGIFFGSITFMYFKPPSSNNMEQEKVSSVFYTTVIPMLNPLIYSLRNKDVKTALKKM 301

Query: 304 L 304
           +
Sbjct: 302 V 302
```

The GPCR5b amino acid sequence has 153 of 306 amino acid residues (50%) identical to, and 198 of 306 residues (64%) positive with, the 309 amino acid M71 from *Mus musculus* (GENBANK-ID:AAG29379.1) (SEQ ID NO:54) (Table 5K). The residue that differs between GPCR5a, GPCR5b and GPCR5c are highlighted in black and marked with the (o) symbol.

Table 5K. BLASTX of GPCR5b against M71

```
>gb|AAG29379.1|AF281061_1 (AF281061) odorant receptor M71 [Mus musculus]
(SEQ ID NO:54) Length = 309
 Score =  290 bits (743), Expect = 1e-77
 Identities = 161/306 (53%), Positives = 206/306 (67%), Gaps = 2/306 (0%)

Query: 1   MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMY 60
            |+  | | +|||||  |||+ ||||  || |||  | ||  |||||| |+ |+|+||||||
Sbjct: 1   MTAENQSTVTEFILGGLTNRPELQLPLFLLFLGIYVVTMVGNLGMITLIGLNSQLHTPMY 60

Query: 61  FFLTNLAFVDLCYTSNATPQMSTNIVSEKT-ISFAGCFTQCYIFIALLLTEFYMLAAMAY 119
            |||+||+ |||||+|  ||+|  | ||++  ||+ || +| | |+  ++ | |||  |||
Sbjct: 61  FFLSNLSLVDLCYSSVITPKMLINFVSQRNLISYVGCMSQLYFFLVFVIAECYMLTVMAY 120

Query: 120 DRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFCRSSVINHFYCA 179
            ||||||  || |++  |  +|  |  | |  |       +  |  +| +|   +|+|++|
Sbjct: 121 DRYVAICQPLLYNIIMSPALCSLLVAFVYAVGLIGSAIETGLMLKLNYCED-LISHYFCD 179

Query: 180 DPPLIKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYAFILAAILRIKSAEGRHKAFST 239
              ||+|||||  ||  | |+|  |||++  +   ||+|||||++|||| | ||| |||||
Sbjct: 180 ILPLMKLSCSSTYDVEMAVFFLAGFDIIVTSLTVLISYAFILSSILRISSNEGRSKAFST 239

Query: 240 CGSHMMAVTLFYGTLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQA 299
            | ||  || ||||+   ||++| |  ++ +  + +|||| | |+ ||||||||||+|| |
Sbjct: 240 CSSHFAAVGLFYGSTAFMYLKPSTASSLAQENVASVFYTTVIPMFNPLIYSLRNKEVKTA 299

Query: 300 LKNVLR 305
            |   ||
Sbjct: 300 LDKTLR 305
```

A multiple sequence alignment is given in Table 5L, with GPCR5a being shown on line 1, in a ClustalW analysis comparing GPCR5a with related protein sequences.

Table 5L. Information for the ClustalW proteins:

1. Novel Human_OLF, GPCR5a, SEQ ID NO:14
2. *Homo sapiens* OLF, SWISSPROT-Acc # Q13606, SEQ ID NO:33
3. *Hylobates lar* (Common Gibbon) OLF, SPTREMBL-Acc # O77758, SEQ ID NO:43
4. *Rattus norvegicus* OLF, SPTREMBL-Acc # Q63394, SEQ ID NO:55

```
GPCR5a          --MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTP
HUMAN_OLF       MEFTDRNYTLVTEFILLGFPTRPELQIVLFLMFLTLYAIILIGNIGLMLLIRIDPHLQTP
GIBBON_OLF      --MANENYTKVTEFIFTGLNYNPQLQVFLFLLFLTFYVISVTGNFGMIVLIRMDSRLHTP
RAT_OLF         --MSVANESISREFILLGFSDRPWLELPLFVVFLVSYIILTIFGNMMIILVSRLDSKLHTP

GPCR5a          MYFFLTNLAFVDLCYTSNATPQMSTNIVS-EKTISFAGCFTQCYIFIALLLTEFYMLAAM
HUMAN_OLF       MYFFLSNLSFVDLCYFSDIVPKMLVNFLSENKSISYYGCALQFYFFCTFADTESFILAAM
GIBBON_OLF      MYFFLSHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASM
RAT_OLF         MYFFLTNLSLLDLCYTTSTVPQMLINICSTRKVISYGGCVVQLFIFLSLGSTECFLLGVM

GPCR5a          AYDRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFCRSNVINHFY
HUMAN_OLF       AYDRYVAICNPLLYTVVMSRGICMRLIVLSYLGGNMSSLVHTSFAFILKYCDKNVINHFF
GIBBON_OLF      AYDRYVAICNPLLYSVFMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGLNVINHFF
RAT_OLF         ALDRFLAICRPLHYSVIMHQRRCLHLAAACWISGFSNSVLQSTWTLQMPLCGHKEVDHFF

GPCR5a          CADPPLIKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYAFILAAILRIKSAEGRHKAF
HUMAN_OLF       CDLPPLLKLSCTDTTINEWLLSTYGSSVEICFIIIISYFFILLSVLKIRSFSGRKKTF
GIBBON_OLF      CDMSPLLSLVCADTRLNKLAVFIMAGAVGVFSGLTILISYIYILMAILRIRSADGRCKTF
RAT_OLF         CEVPALLKLSCVDTTANEAELFFISVLFLLIPVTLILISYAFIVQAVLKIRSAECRRKAF

GPCR5a          STCGSHMMAVTLFYGTLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVK
HUMAN_OLF       STCASHLTSVTIYYGTLLEIYSRPSYLYSPNTDKIISVFYTIFIPVLNPLIYSLRNKDVK
GIBBON_OLF      STCSSHLTAVFILYGTLFFIYVRPSASFPLDLNKVVSVFYTAVIPMLNPLIYSLRNKEVK
RAT_OLF         GTCCSHLIVVVLFYGTAIYMYLQPPSPSSKDRGKMVSLFYGIITPMLNPLIYTLRNEEVK

GPCR5a          QALKNVLR--------
```

```
HUMAN_OLF       DAAEKVLRSKVDSS-
GIBBON_OLF      DAIHRTVTQRKFCKA
RAT_OLF         GAFKRLMKRIILIGK
```

The presence of identifiable domains in GPCR5 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/interpro/). The results indicate that this protein contains the following protein domains (as defined by Interpro) at the indicated positions: domain name 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family) at amino acid positions 41 to 289. This indicates that the sequence of GPCR5 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

DOMAIN results for GPCR5a were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 5M with the statistics and domain description. Residues 1-180 (SEQ ID NO:29) and residues 313-377 (SEQ ID NO:37) of 7tm_1 are aligned with GPCR4 in Table 4O. The residue that differs between GPCR5a, GPCR5b and GPCR5c are highlighted in black and marked with the (o) symbol.

Table 5M. DOMAIN results for GPCR5a.

```
Sbjct: 7 transmembrane receptor (rhodopsin family) fragment (SEQ ID NO: 29)
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family), 377 aa
 Statistics for GPCR5a:    Score = 92.8 bits (229), Expect = 2e-20
 Statistics for GPCR5b:    Score = 92.8 bits (229), Expect = 2e-20

Query: 41   GNLGMIMLMRLDSRLHTPMYFFLTNLAFVDLCYTSNATPQMSTNIVS-EKTISFAGCFTQ  99
Sbjct: 1    GNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF  60
             **: : * :  :  * *   : : :**  **   :   * :    *  *   *   *

Query: 100  CYIFIALLLTEFYMLAAMAYDRYVAIYDPLRYS-VKTSRRVCICLATFPYVYGFSDGLFQ  158
Sbjct: 61   VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVWVLSFTISCPM  120
               : : :       * *:: *** *:  *: *:   :*:*    :    :*  *:
                          o
Query: 159  AILTFRLTFCRSNVINHFYCADPPLIKLSCSDTYVKEHAMFISAGFNLSSSLTIVLVSYA  218
Sbjct: 121  LFGLNNTDQN------------------ECIIANPAFVVYSSIVSFYV--PFIVTLLVYI  160
                                       *                * :     : *: *
                           o
Query: 219  FIL-AAILRIKSAEGRHKAF  237
Sbjct: 161  KIYIVLRRRRKRVNTKRSSR  180
               *      * *    :  :

Sbjct: 7 transmembrane receptor (rhodopsin family) fragment (SEQ ID NO: 37)
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family), 377 aa
  Statistics for GPCR5a: Score = 35.8 bits (81), Expect = 0.003
  Statistics for GPCR5b:  Score = 35.8 bits (81), Expect = 0.003
                 o
Query: 226  RIKSAEGRHKAFSTCGSHMMAVTLFYGTLFCMYIRP-PTDKTVEESKIIAVFYTFVSPVL  284
Sbjct: 313  KLSQQKEKKATQMLAIVLGVFIICWLPFFITHILNIHCDCNIPPVLYSAFTWLGYVNSAV  372
             :    : :          : :  :        :                  :  :*:  :

Query: 285  NPLIY  289
Sbjct: 373  NPIIY  377
             **:**
```

GPCR5 is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to Public EST sources, Literature sources, and/or RACE sources.

In the following positions, one or more consensus positions of GPCR5 have been identified as single nucleotide polymorphisms ("SNPs"). As shown in Table 5N, "Depth" represents the number of clones covering the region of the SNP. The Putative Allele Frequency (Putative Allele Freq.) is the fraction of all the clones containing the SNP. A dash ("-"), when shown, means that a base is not present. The sign ">" means "is changed to".

Table 5N: GPCR5 Single Nucleotide Polymorphisms

Consensus Position	Depth	Change	Putative Allele Freq
82	23	T > A	0.478
587	30	T > C	0.067
597	32	G > A	0.375

The protein similarity information, expression pattern, and map location for the GPCR5 protein and nucleic acid disclosed herein suggest that GPCR5 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody),

(iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon.

The nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications implicated in various GPCR- or OR-related diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: developmental diseases, MHCII and III diseases (immune diseases), Taste and scent detectability Disorders, Burkitt's lymphoma, Corticoneurogenic disease, Signal Transduction pathway disorders, Retinal diseases including those involving photoreception, Cell Growth rate disorders; Cell Shape disorders, Feeding disorders;control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of apetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; Albright Hereditary Ostoeodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidoluysian atrophy (DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like. The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding GPCR5 may be useful in gene therapy, and GPCR5 may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Ostoeodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and

dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding GPCR5, and the GPCR5 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods and other diseases, disorders and conditions of the like. Other GPCR-related diseases and disorders are contemplated.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated epitope of GPCR5c would be from amino acid 10 to 50. In another embodiment, a contemplated epitope of GPCR5c would be from amino acid 35 to 45. In yet another embodiment, a contemplated epitope of GPCR5c would be from amino acid 80 to 120. In yet another embodiment, a contemplated epitope of GPCR5c would be from amino acid 135 to 160. In yet another embodiment, a contemplated epitope of GPCR5c would be from amino acid 205 to 235. In yet another embodiment, a contemplated epitope of GPCR5c would be from amino acid 245 to 260. In yet another embodiment, a contemplated epitope of GPCR5c would be from amino acid 275 to 290.

GPCR6

The novel nucleic acid of 1050 nucleotides GPCR6 (also designated AP001112_D) encoding a novel Olfactory Receptor-like protein is shown in Table 6A. An ORF was identified beginning with an ATG initiation codon at nucleotides 53-55 and ending with a TAA codon at nucleotides 1007-1009. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 6A, and the start and stop codons are in bold letters.

Table 6A GPCR6 Nucleotide Sequence (SEQ ID NO:19)

TGTAATGGACTTCTCATTCACCTTGATTTATTTTCATCCATTTAAGTGAAAA**ATG**TTGGTACCTAAGAAAATGGTTAGAGGAAA
TTCTACTTTGGTGACGGAATTTATTCTCTTGGGATTAAAGGATCTTCCAGAGCTTCAGCCCATCCTCTTTGTACTGTTCCTGCT
AATCTACCTGATCACTGTCGGGGGGAACCTTGGGATGTTGGTGTTGATCAGGATAGATTCACGCCTCCACACCCCCATGTATTT
CTTTCTTGCTAGTTTGTCCTGCTTGGATTTGTATTACTCCACTAATGTGACTCCCAAGATGTTGGTGAACTTCTTCTCAGACAA
GAAAGCCATTTCCTATGCTGCTTGTTTAGTCCAGTGCTATTTTTTCATTGCTGTGGTGATTACTGAATATTATATGCTAGCTGT
AATGGCCTATGATAGGTATGTGGCCATCTGTAACCCTTTGCTTTACAGCAGCAAGATGTCCAAAGGGCTCTGTATTCGCCTGAT
TGCTGGTCCATATGTCTATGGGTTTCTTAGTGGACTGATGGAAACCATGTGGACATACCACTTGACCTTCTGTGGCTCCAATAT
CATTAATCACTTCTACTGTGCTGACCCACCCCTCATCCGACTTTCCTGCTCTGACACTTTCATTAAGGAAACATCCATGTTTGT
GGTAGCATGTTTTAACCTCTCCAGCTCCCTCATCATAATCCTCATCTCCTACATCTTCATTCTCATTGCCATCCTGAGGATGCG
TTCTGCTGAAAGTAGGCGCAAAGCGTTCTCCACCTGCGGGTCCCACCTGGTGGCAGTGACTGTGTTTTATGGAACCCTGTTCTG

CATGTACGTTAGACCTCCCACGGACAGGTCAGTGGAACAGTCCAAAGTCATTGCTGTTTTCTACACTTTTGTAAGCCCTATGTT
GAACCCCATCATCTATAGTTTGAGGAACAAGGATGTGAAACAAGCTTTTTGGAAACTGATCAGAAGAAACGTGCTTTTGAAGTA
AAATCAGTGTATCTTTATTAGTCAAATAAAAAAATCTTTCTA

T - sequence change from A to T to correct a stop codon.

The GPCR6 protein encoded by SEQ ID NO:19 has 318 amino acid residues and is presented using the one-letter code in Table 6B.

Table 6B. Encoded GPCR6 protein sequence (SEQ ID NO:20).

MLVPKKMVRGNSTLVTEFILLGLKDLPELQPILFVLFLLIYLITVGGNLGMLVLIRIDSRLHTPMYFFLASLSCLDLYYSTNVT
PKMLVNFFSDKKAISYAACLVQCYFFIAVVITEYYMLAVMAYDRYVAICNPLLYSSKMSKGLCIRLIAGPYVYGFLSGLMETMW
TYHLTFCGSNIINHFYCADPPLIRLSCSDTFIKETSMFVVACFNLSSSLIIILISYIFILIAILRMRSAESRRKAFSTCGSHLV
AVTVFYGTLFCMYVRPPTDRSVEQSKVIAVFYTFVSPMLNPIIYSLRNKDVKQAFWKLIRRNVLLK

In a search of sequence databases, it was found, for example, that the GPCR6 nucleic acid sequence has 621 of 939 bases (66 %) identical to a and 621 of 939 bases (66%) positive with *Gallus gallus* species Olfactory Receptor 2 (GENBANK-ID: X94742) (Table 6C).

Table 6C. BLASTN of GPCR6 against OR2 (SEQ ID NO:51)

```
>gb:GENBANK-ID:GGCOR2GEN|acc:X94742 G. gallus cor2 DNA for olfactory receptor 2
Gallus gallus, (SEQ ID NO:51) 996 bp.
Score = 1539 (230.9 bits), Expect = 1.8e-63, P = 1.8e-63
Identities = 621/939 (66%), Positives = 621/939 (66%), Strand = Plus / Plus

Query:    67 GAAAATGGTTAGAGGAAATTCTACTTTGGTGACGGAATTTATTCTCTTGGGATTAAAGGA 126
             ||  ||||  |  ||||||   |  |   | || |||||| | ||||||||||||     ||
Sbjct:    54 GATGATGGCCAAGGGAAATCACAGCTCCATCACTGAATTTGTGCTCTTGGGATTCTCTGA 113

Query:   127 TCTTCCAGAGCTTCAGCCCATCCTCTTTGTACTGTTCCTGCTAATCTACCTGATCACTGT 186
                 |   | ||| |  | |||||| |    ||| |||| |||||||||||||||| |
Sbjct:   114 AAAGAGGGCCATCCAGGCTGTTCTCTTTATGGGCTTCTTGCTGATCTACCTGATCACTCT 173

Query:   187 CGGGGGGAACCTTGGGATGTTGGTGTTGATCAGGATAGATTCACGCCTCCACACCCCCAT 246
               || ||  | || ||| |    |||||||||| | || || || || || |||||||| ||
Sbjct:   174 GCTAGGCAATGTGGGCATGATCACATTGATCAGGCTGGACTCCCGGCTTCACACCCCTAT 233

Query:   247 GTATTTCTTTCTT-GCTAGTTTGTCCTGCTTGGAT-T-TG-TATTACTCCACTAATGTGA 302
             ||| ||||| ||  || || ||||||| | | ||| | || |||| |||||| |||   |
Sbjct:   234 GTACTTCTTCCTGAGC-AGCTTGTCCTTCCTCGATATCTGCTATTCCTCCAC-AATC--A 289

Query:   303 CTCC-CAAGATGTTGGTGA-ACTTCTTCTCAGACA-AGAAAGCCATTTCCTATGCTGCTT 359
             |||| | || || | | | || ||    ||  || |||||| ||||||| |  |||| |
Sbjct:   290 CTCCTCGAG-TGCTC-TCAGACCTCCCAGCAT-CACAGAAAGTCATTTCCCACTCTGCAT 346

Query:   360 GTTTAGTCCAGTGCTATTTTTTCATTG-CTGTGGTGATTACTGAATATTATATGCTAGCT 418
             |  | |  |||| ||||| | |  || || ||   |  || || |  ||| |  | ||
Sbjct:   347 GCCTGGCACAGTTTTATTTCTACGCTGTCTTTGCC-ACCACAGAGTGCTATCTTTTGGCC 405

Query:   419 GTAATGGCCTATGATAGGTATGTGGCCATCTGTAACCCTTTGCTTTACAGCAGCAAGATG 478
             | |||||| |||||  | || |||||||||||| | |||| |||| ||   |  |  |||
Sbjct:   406 GCAATGGCATATGACCGCTACGTGGCCATCTGCAGCCCTCTGCTCTATGTCTTCTCCATG 465

Query:   479 TCCAA-AGGGCTCTGTATTCGCCTGATTGCTGGTCCATATGTCTAT-GGGTTTCTTAGTG 536
             ||||  || | | ||| | |  ||| |||||||  ||||  | | | ||| || | | ||
Sbjct:   466 TCCAGCAGAGTT-TGTGTGCTGCTGGTTGCTGGCTCATACCT-TGTCGGGGTTGTGAATG 523

Query:   537 G-ACTGATGGAAACCATG-TGGACATACCACTTGACCTTCTGTGGCTCCAATATCATTAA 594
               ||  ||  | ||   | | | ||   ||  || ||||||||||  |||| ||||| ||
Sbjct:   524 CCACC-ATTCACACAGGGCTTG-CACTGCAGCTGTCCTTCTGTGGTCCCAACATCATCAA 581
```

```
Query:   595 TCACTTCTACTGTG-CTGACCCACCCCTCATCCGACTT-TCCTGCTCTGACACTTTCATT 652
Sbjct:   582 TCACTTCTACTGTGACGGTCCC-CCGCTC-TACGCCATCTCGTGCACAGACCCCACCACC 639

Query:   653 AAGGAAACATCCATGTTTGTGGTAGCATGATTTAACCT-CTCCAGCTCCCTCATCATAAT 711
Sbjct:   640 AACGAGATTGCGATATTTCTTGTGGTTGGCTTCAACATGCTC-ATCACCAGCGTGACCAT 698

Query:   712 CCTCATCTCCTACATCTTCATTCTCATTGCCATCCTGAGGATGCGTTCTGCTGAAAGTAG 771
Sbjct:   699 CTTCATCTCCTACACCTACATCCTGTTCGCTGTCCTCAGGATGCACACAGCTGCAGGCAA 758

Query:   772 GCGCAAAGCGTTCTCCACCTGCGGGTCCCACCTGGTGGCAGTGACTGTGTTTTATGGAAC 831
Sbjct:   759 ACGCAAAACCTTCTCCACGTGTGCGTCCCACCTGGCCACCGTCACCCTATTCTATGCCTC 818

Query:   832 C-CTGTTCTGCATGTACGTTA--GACCTCCCACGGACAGGTCAGTGGAACAGTCCAAAGT 888
Sbjct:   819 TGCTGGT-TCCATGTAC-TCACGGCCCAGCTCCAGGCAC-TCCCAGGACCTGGACAAGGT 875

Query:   889 CATTGCTGTTTTCTACACTTTTGTAAGCCCTATGTTGAACCCCATCATCTATAGTTTGAG 948
Sbjct:   876 GGCCTCTGTGTTCTACACCATGGTGACCCCCATGCTGAACCCCCTCATCTACAGCCTGAG 935

Query:   949 GAACAAGGATGTGAAACAAGCTTTTTGGAAACTGATCAGAAGAAAC-GTGCTTTTGA 1004
Sbjct:   936 GAACCAGGAGGTAAAGGATGTTTTAGGGAAAGTGATGGGGAGGAAGAGTGTCTCTGA 992
```

The GPCR6 amino acid has 165 of 307 amino acid residues (53 %) identical to, and 226 of 307 residues (73%) positive with, the 312 amino acid OR4 from *Gallus gallus* (ptnr: SPTREMBL-ACC: O77756) (SEQ ID NO:56) (Table 6D).

Table 6D. BLASTX of GPCR6 against OR4

```
>ptnr:SPTREMBL-ACC:Q90808 OLFACTORY RECEPTOR 4 - Gallus gallus (Chicken), 312 aa
(fragment). (SEQ ID NO:56)
Score = 867 (305.2 bits), Expect = 7.0e-86, P = 7.0e-86
Identities = 165/307 (53%), Positives = 226/307 (73%), Frame = +2

Query:    71 MVRGNSTLVTEFILLGLKDLPELQPILFVLFLLIYLITVGGNLGMLVLIRIDSRLHTPMY 250
Sbjct:     1 MAEGNHTLASEFILVGLSDHPKMKAALFVVFLLIYVITFQGNLGIIILIQGDPRLHTSMY 60

Query:   251 FFLASLSCLDLYYSTNVTPKMLVNFFSDKKAISYAACLVQCYFFIAVVITEYYMLAVMAY 430
Sbjct:    61 FFLSSLSVVDICFSSVIAPRTLVNFLSERRTISFTGCTGQTFFYIVFVTTECFLLAVMAY 120

Query:   431 DRYVAICNPLLYSSKMSKGLCIRLIAGPYVYGFLSGLMETMWTYHLTFCGSNIINHFYCA 610
Sbjct:   121 DRYVAICNPLLYSTIMTRRQCMQLVVGSYIGGILNAIIQTTFIIRLPFCGSNIINHFFCD 180

Query:   611 DPPLIRLSCSDTFIKETSMFVVA*FNLSSSLIIILISYIFILIAILRMRSAESRRKAFST 790
Sbjct:   181 VPPLLALSLASTYISEMILFSLAGIIELSTVTSILVSYIFISCAILRIRSAEGRQKALST 240

Query:   791 CGSHLVAVTVFYGTLFCMYVRPPTDRSVEQSKVIAVFYTFVSPMLNPIIYSLRNKDVKQA 970
Sbjct:   241 CASHLTAVTLLYGTTIFTYLRPSSSYSLNTDKVVSVFYTVVIPMLNPLIYSLRNQEVKGA 300

Query:   971 FWKLIRR 991
Sbjct:   301 LSRVVER 307
```

The GPCR6 amino acid has 153 of 313 amino acid residues (48%) identical to, and 199 of 313 residues (62%) positive with, the 314 amino acid OR93Ch from *Pan troglodytes* OR93Ch (GENBANK-ID:AAC63969.1) (SEQ ID NO:57) (Table 6E).

Table 6E.-BLASTX of GPCR6 against OR93Ch

```
>gb|AAC63969.1| (AF045577) olfactory receptor OR93Ch [Pan troglodytes]
(SEQ ID NO:57) Length = 314
 Score =  293 bits (749), Expect = 2e-78
 Identities = 169/313 (54%), Positives = 215/313 (69%), Gaps = 1/313 (0%)

Query: 7   MVRGNSTLVTEFILLGLKDLPELQPILFVLFLLIYLI-TVGGNLGMLVLIRIDSRLHTPM 65
           |   | | |||||  ||   |+||  || |||       | |||||+|||||||||||||
Sbjct: 1   MANENYTKVTEFIFTGLNYNPQLQVFLFLLFLTTFYVINVTGNLGMIVLIRIDSRLHTPM 60

Query: 66  YFFLASLSCLDLYYSTNVTPKMLVNFFSDKKAISYAACLVQCYFFIAVVITEYYMLAVMA 125
           ||||+ || +|+ +|+ |+|||| +||  +||||+  | +| +||   |  | ++|| ||
Sbjct: 61  YFFLSHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMA 120

Query: 126 YDRYVAICNPLLYSSKMSKGLCIRLIAGPYVYGFLSGLMETMWTYHLTFCGSNIINHFYC 185
           ||||||||||||||  ||+ |||+|+ |||| | ++ +  |   + | ||| |+||||+|
Sbjct: 121 YDRYVAICNPLLYSVAMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGPNVINHFFC 180

Query: 186 ADPPLIRLSCSDTFIKETSMFVVACFNLSSSLIIILISYIFILIAILRMRSAESRRKAFS 245
              ||+ | |+|| + + ++|+||       |   |||||| || ||||+|||+ | | ||
Sbjct: 181 DMSPLLSLVCADTRLNKLAVFIVAGAAGVFSGLTILISYIYILMAILRIRSADGRCKTFS 240

Query: 246 TCGSHLVAVTVFYGTLFCMYVRPPTDRSVEQSKVIAVFYTFVSPMLNPIIYSLRNKDVKQ 305
           || ||| || + ||||| +|||||     |++ +|+++|||| | |||||+|||||||+||
Sbjct: 241 TCSSHLTAVFILYGTLFFIYVRPSASFSLDLNKLVSVFYTAVIPMLNPLIYSLRNKEVKD 300

Query: 306 AFWKLIRRNVLLK 318
           |  + + +    |
Sbjct: 301 AIHRTVTQRKFCK 313
```

The GPCR6 amino acid has 150 of 312 amino acid residues (48%) identical to, and 198 of 312 residues (63%) positive with, the 313 amino acid OR93Gib from *Hylobates lar* (GENBANK-ID:AAC63971.1) (SEQ ID NO:58) (Table 6F).

Table 6F.-BLASTX of GPCR6 against OR93Gib

```
>gb|AAC63971.1| (AF045580) olfactory receptor OR93Gib [Hylobates lar]
(SEQ ID NO:58) Length = 313
 Score =  291 bits (745), Expect = 7e-78
 Identities = 168/312 (54%), Positives = 216/312 (69%)

Query: 7   MVRGNSTLVTEFILLGLKDLPELQPILFVLFLLIYLITVGGNLGMLVLIRIDSRLHTPMY 66
           |   | | |||||  ||   |+||  || |||  | |+| || ||+||||+|||||||||
Sbjct: 1   MANENYTKVTEFIFTGLNYNPQLQVFLFLLFLTFYVISVTGNFGMIVLIRMDSRLHTPMY 60

Query: 67  FFLASLSCLDLYYSTNVTPKMLVNFFSDKKAISYAACLVQCYFFIAVVITEYYMLAVMAY 126
           |||+ || +|+ +|+ |+|||| +||  +||||+  | +| +||   |  | ++|| |||
Sbjct: 61  FFLSHLSFVDICFSSVVSPKMLTDFFVKRKAISFLGCALQQWFFGFFVAAECFLLASMAY 120

Query: 127 DRYVAICNPLLYSSKMSKGLCIRLIAGPYVYGFLSGLMETMWTYHLTFCGSNIINHFYCA 186
           ||||||||||||||  ||+ |||+|+ |||| | ++ +  |   + | ||| |+||||+|
Sbjct: 121 DRYVAICNPLLYSVFMSQRLCIQLVVGPYVIGLMNTMTHTTNAFRLPFCGLNVINHFFCD 180

Query: 187 DPPLIRLSCSDTFIKETSMFVVACFNLSSSLIIILISYIFILIAILRMRSAESRRKAFST 246
              ||+ | |+|| + + ++|++|       |    ||||| || ||||+|||+ | | |||
Sbjct: 181 MSPLLSLVCADTRLNKLAVFIMAGAVGVFSGLTILISYIYILMAILRIRSADGRCKTFST 240
```

```
Query: 247 CGSHLVAVTVFYGTLFCMYVRPPTDRSVEQSKVIAVFYTFVSPMLNPIIYSLRNKDVKQA 306
           | ||| || + ||||| +|||| ++ +||++|||| | |||||+|||||||+|| |
Sbjct: 241 CSSHLTAVFILYGTLFFIYVRPSASFPLDLNKVVSVFYTAVIPMLNPLIYSLRNKEVKDA 300

Query: 307 FWKLIRRNVLLK 318
             + + +    |
Sbjct: 301 IHRTVTQRKFCK 312
```

The GPCR6 amino acid has 143 of 307 amino acid residues (46%) identical to, and 193 of 307 residues (62%) positive with, the 332 amino acid OR2 from *Gallus gallus* (embCAA64368.1) (SEQ ID NO:59) (Table 6G).

Table 6G.-BLASTX of GPCR6 against OR2

```
>emb|CAA64368.1| (X94742) olfactory receptor 2 [Gallus gallus] (SEQ ID NO:59), 332
aa
 Score =  290 bits (743), Expect = 1e-77
 Identities = 160/307 (52%), Positives = 210/307 (68%)

Query: 7   MVRGNSTLVTEFILLGLKDLPELQPILFVLFLLIYLITVGGNLGMLVLIRIDSRLHTPMY 66
           | +|| + +|||+|||  +   +|  ||  ||||||||+ ||+||+ |||+||||||||||
Sbjct: 20  MAKGNHSSITEFVLLGFSEKRAIQAVLFMGFLLIYLITLLGNVGMITLIRLDSRLHTPMY 79

Query: 67  FFLASLSCLDLYYSTNVTPKMLVNFFSDKKAISYAACLVQCYFFIAVVITEYYMLAVMAY 126
           |||+|||  ||+ ||+ +||++| +  + +| ||++||| | ||+     || |+|| |||
Sbjct: 80  FFLSSLSFLDICYSSTITPRVLSDLPASQKVISHSACLAQFYFYAVFATTECYLLAAMAY 139

Query: 127 DRYVAICNPLLYSSKMSKGLCIRLIAGPYVYGFLSGLMETMWTYHLTFCGSNIINHFYCA 186
           |||||||+||||   ||  +|+ |+|| |+ | ++  + |     |+||| ||||||||
Sbjct: 140 DRYVAICSPLLYVFSMSSRVCVLLVAGSYLVGVVNATIHTGLALQLSFCGPNIINHFYCD 199

Query: 187 DPPLIRLSCSDTFIKETSMFVVACFNLSSSLIIILISYIFILIAILRMRSAESRRKAFST 246
            |||  +||+|    | ++|+|  ||       | |||| || | ||| +|  +|| |||
Sbjct: 200 GPPLYAISCTDPTTNEIAIFLVVGFNMLITSVTIFISYTYILFAVLRMHTAAGKRKTFST 259

Query: 247 CGSHLVAVTVFYGTLFCMYVRPPTDRSVEQSKVIAVFYTFVSPMLNPIIYSLRNKDVKQA 306
           | |||  ||+|| +  || || +  | +  || +|||| |+|||||+|||||||++||
Sbjct: 260 CASHLATVTLFYASAGSMYSRPSSRHSQDLDKVASVFYTMVTPMLNPLIYSLRNQEVKDV 319

Query: 307 FWKLIRR 313
             |++ |
Sbjct: 320 LGKVMGR 326
```

The GPCR6 amino acid has 150 of 311 amino acid residues (48%) identical to, and 193 of 311 residues (61%) positive with, the 311 amino acid K30 from *Mus musculus* (GENBANK-ID:AAG39871.1) (SEQ ID NO:60) (Table 6H).

Table 6H.-BLASTX of GPCR6 against K30

```
>gb|AAG39871.1|AF282286_1 (AF282286) odorant receptor K30 [Mus musculus]
 (SEQ ID NO:60) Length = 311
 Score =  290 bits (743), Expect = 1e-77
 Identities = 166/311 (53%), Positives = 206/311 (66%)

Query: 7   MVRGNSTLVTEFILLGLKDLPELQPILFVLFLLIYLITVGGNLGMLVLIRIDSRLHTPMY 66
           |++|| + |||||| || + ||||  || ||| ||  || |||||++|| + | ||||||
Sbjct: 1   MLKGNLSEVTEFILAGLTNKPELQLPLFLLFLAIYVVTVVGNLGMIILILLSSHLHTPMY 60

Query: 67  FFLASLSCLDLYYSTNVTPKMLVNFFSDKKAISYAACLVQCYFFIAVVITEYYMLAVMAY 126
           +||+||| +||  || + ||||||| + |  |||  |+ | |||+   | | +|||||||
Sbjct: 61  YFLSSLSFIDLCQSTVIIPKMLVNFVTVKNIISYPECMTQLYFFVTFAIAECHMLAVMAY 120

Query: 127 DRYVAICNPLLYSSKMSKGLCIRLIAGPYVYGFLSGLMETMWTYHLTFCGSNIINHFYCA 186
```

```
            ||||||||||||++ ||  +|  +| | |    +     |+    + || +|+|||++|
Sbjct: 121 DRYVAICNPLLYNAVMSFQVCSSMIFGVYSIALIGATTHTVCMLRVNFCKANVINHYFCD 180

Query: 187 DPPLIRLSCSDTFIKETSMFVVACFNLSSSLIIILISYIFILIAILRMRSAESRRKAFST 246
              ||+ | |||||| |  +   + ||       || |||||   ||+++| | | |||||
Sbjct: 181 LFPLLELPCSDTFINEVVVLCFSVFNIFIPTLTILTSYIFIIASILQIKSTEGRSKAFST 240

Query: 247 CGSHLVAVTVFYGTLFCMYVRPPTDRSVEQSKVIAVFYTFVSPMLNPIIYSLRNKDVKQA 306
            | ||+ || +|+|+|+  ||++| +  |++| || +|||| | |||||+||||||||||| |
Sbjct: 241 CSSHISAVAIFFGSLAFMYLQPSSVSSMDQGKVSSVFYTIVVPMLNPLIYSLRNKDVKVA 300

Query: 307 FWKLIRRNVLL 317
              |   |   |
Sbjct: 301 LNKFFERKFFL 311
```

The GPCR6 amino acid has 149 of 311 amino acid residues (47%) identical to, and 192 of 311 residues (60%) positive with, the 314 amino acid K11 from *Mus musculus* (GENBANK-ID:AAG39856.1) (SEQ ID NO:61) (Table 6I).

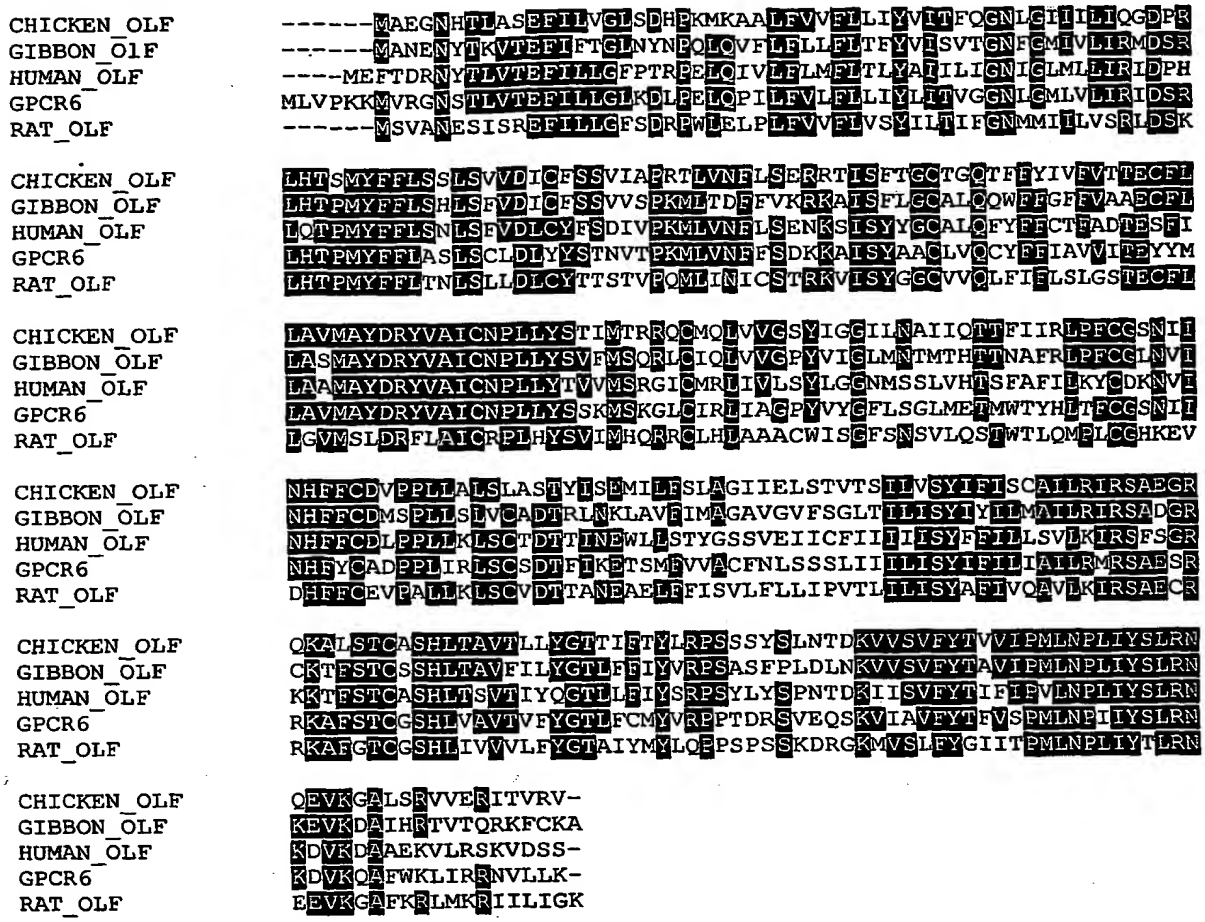
Table 6I. BLASTX of GPCR6 against K11 (SEQ ID NO:61)

```
>gb|AAG39856.1|AF282271_1 (AF282271) odorant receptor K11 [Mus musculus]
 (SEQ ID NO:61), 314 aa
 Score =  289 bits (739), Expect = 4e-77
 Identities = 164/311 (53%), Positives = 207/311 (66%)

Query: 7   MVRGNSTLVTEFILLGLKDLPELQPILFVLFLLIYLITVGGNLGMLVLIRIDSRLHTPMY 66
            |  ||   |||| | || + ||||  || ||  || ||| |||||++|| + | ||||||
Sbjct: 4   MTSGNYCTVTEFFLAGLSEKPELQLPLFFLFIGIYMITVAGNLGMIILIGLSSHLHTPMY 63

Query: 67  FFLASLSCLDLYYSTNVTPKMLVNFFSDKKAISYAACLVQCYFFIAVVITEYYMLAVMAY 126
            +||+||| +|   || |||||||| ++|  ||| |+ | |||+   | | |+|| |||
Sbjct: 64  YFLSSLSFIDFCQSTVVTPKMLVNFVTEKNIISYPGCMTQLYFFLIFAIAECYILAAMAY 123

Query: 127 DRYVAICNPLLYSSKMSKGLCIRLIAGPYVYGFLSGLMETMWTYHLTFCGSNIINHFYCA 186
            ||||||||||||+  ||  + | ||+| |+ | +     | +   + ||  ++|||++|
Sbjct: 124 DRYVAICNPLLYNVTMSYQIYIFLISGVYIIGVICASAHTGFMVRIRFCKLDVINHYFCD 183

Query: 187 DPPLIRLSCSDTFIKETSMFVVACFNLSSSLIIILISYIFILIAILRMRSAESRRKAFST 246
              ||++|+||+|+| |  +      |       |  |||||   |||+|| | | |||||
Sbjct: 184 LLPLLKLACSNTYINEMLILFFGTLNIFVPILTIITSYIFIIASILRIRSTEGRSKAFST 243

Query: 247 CGSHLVAVTVFYGTLFCMYVRPPTDRSVEQSKVIAVFYTFVSPMLNPIIYSLRNKDVKQA 306
            | ||++|| ||+|+|  ||++| +  |++| || +|||| | |||||+|||||||||  |
Sbjct: 244 CSSHILAVAVFFGSLAFMYLQPSSVSSMDQGKVSSVFYTIVVPMLNPLIYSLRNKDVAVA 303

Query: 307 FWKLIRRNVLL 317
              |+| |   +
Sbjct: 304 LKKIIERKTFM 314
```

A multiple sequence alignment is given in Table 6J, with the GPCR6 protein being shown on line 4, in a ClustalW analysis comparing GPCR6 with related protein sequences.

Table 6J. Information for the ClustalW proteins:

1. *Gallus gallus* (CHICKEN) OLF, SPTREMBL-Acc # Q90808, SEQ ID NO:56
2. *Hylobates lar* (Common Gibbon) OLF, SPTREMBL-Acc # O77758, SEQ ID NO:43
3. *Homo sapiens* OLF, SWISSPROT-Acc # Q13606, SEQ ID NO:33
4. Novel Human_OLF, GPCR6, SEQ ID NO:20
5. *Rattus norvegicus* OR, Acc # G264617, SEQ ID NO:62



DOMAIN results for GPCR6 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 6K with the statistics and domain description. Residues 1-158 (SEQ ID NO:29) and residues 313-377 (SEQ ID NO:37) of 7tm_1 are aligned with GPCR4 in Table 6K.

Table 6K. DOMAIN results for GPCR6.

```
Sbjct: 7 transmembrane receptor (rhodopsin family) fragment (SEQ ID NO:29)
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family)
           Length = 377
           Score = 95.1 bits (235), Expect = 5e-21

Query:  47   GNLGMLVLIRIDSRLHTPMYFFLASLSCLDLYYSTNVTPKMLVNFFSDKKAISYAACLVQ  106
Sbjct:  1    GNVLVCMAVSREKALQTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGEWKFSRIHCDIF  60
             **: : : :  :  * *   : : **:  **  :* * * ::      :   *   * :

Query:  107  CYFFIAVVITEYYMLAVMAYDRYVAICNPLLYSSKM-SKGLCIRLIAGPYVYGFLSGLME  165
Sbjct:  61   VTLDVMMCTASILNLCAISIDRYTAVAMPMLYNTRYSSKRRVTVMIAIVWVLSFTISCPM  120
                : :       *  :: *** *:  *:**::::  **     :**  :*  *

Query:  166  -TMWTYHLTF-CGSNIINHFYCADPPLIRLSCSDTFIKETSMFVVA  209
Sbjct:  121  LFGLNNTDQNECIIANPAFVVY--------SSIVSFYVPFIVTLLV  158
                        *                  *   :*     : ::

Sbjct: 7 transmembrane receptor (rhodopsin family) fragment (SEQ ID NO:37)
gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family)
           Length = 377
```

```
                Score = 38.5 bits (88), Expect = 5e-04

Query:  233  RMRSAESRRKAFSTCGSHLVAVTVFYGTLFCMYVRP-PTDRSVEQSKVIAVFYTFVSPML  291
Sbjct:  313  KLSQQKEKKATQMLAIVLGVFIICWLPFFITHILNIHCDCNIPPVLYSAFTWLGYVNSAV  372
             ::   : ::          * :  :         :                 :  :*:  :

Query:  292  NPIIY  296
Sbjct:  373  NPIIY  377
             *****
```

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor -like protein may be useful in gene therapy, and the olfactory receptor -like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer; Immune response; AIDS; asthma; Crohn's disease; multiple sclerosis; and Albright Hereditary Ostoeodystrophy. Other GPCR-related diseases and disorders are contemplated.

The GPCR6 nucleic acid and protein , or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. This novel protein also has immense value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

GPCR7

The novel nucleic acid of 981 nucleotides GPCR7 (also designated. AP001112_da1) encoding a novel OR-like protein is shown in Table 7A. An ORF begins with an ATG initiation codon at nucleotides 27-29 and ends with a TGA codon at nucleotides 942-944. Putative untranslated regions, if any, are found upstream from the initiation codon and downstream from the termination codon.

Table 7A. GPCR7 Nucleotide Sequence (SEQ ID NO:21)

```
AGCTTGAAGAGCAAACTGTCAGGAATATGTCCAACACAAATGGCAGTGCAATCACAGAATTCATTTTACTTGGGCT
CACAGATTGCCCGGAACTCCAGTCTCTGCTTTTTGTGCTGTTTCTGGTTGTTTACCTCGTCACCCTGCTAGGCAAC
CTGGGCATGATAATGTTAATGAGACTGGACTCTCGCCTTCACACGCCCATGTACTTCTTCCTCACTAACTTAGCCT
TTGTGGATTTGTGCTATACATCAAATGCAACCCCGCAGATGTCGACTAATATCGTATCTGAGAAGACCATTTCCTT
TGCTGGTTGCTTTACACAGTGCTACATTTTCATTGCCCTTCTACTCACTGAGTTTTACATGCTGGCAGCAATGGCC
TATGACCGCTATGTGGCCATATATGACCCTCTGCGCTACAGTGTGAAAACGTCCAGGAGAGTTTGCATCTGCTTGG
```

CCACATTTCCCTATGTCTATGGCTTCTCAGATGGACTCTTCCAGGCCATCCTGACCTTCCGCCTGACCTTCTGTAG
ATCCAATGTCATCAACCACTTCTACTGTGCTGACCCGCCGCTCATTAAGCTTTCTTGTTCTGATACTTATGTCAAA
GAGCATGCCATGTTCATATCTGCTGGCTTCAACCTCTCCAGCTCCCTCACCATCGTCTTGGTGTCCTATGCCTTCA
TTCTTGCTGCCATCCTCCGGATCAAATCAGCAGAGGGAAGGCACAAGGCATTCTCCACCTGTGGTTCCCATATGAT
GGCTGTCACCCTGTTTTATGGGACTCTCTTTTGCATGTATATAAGACCACCAACAGATAAGACTGTTGAGGAATCT
AAAATAATAGCTGTCTTTTACACCTTTGTGAGTCCGGTACTTAATCCATTGATCTACAGTCTGAGGAATAAAGATG
TGAAGCAGGCCTTGAAGAATGTCCTGAGATGAAATATTGTCATGACCATGGTGATGCCTTTGTTTCCTA

The GPCR7 protein encoded by SEQ ID NO:20 has 305 amino acid residues and is presented using the one-letter code in Table 7B. The SignalP, Psort and/or Hydropathy profile for GPCR7 predict that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The SignalP shows a cleavage site between amino acids 44 and 45, *i.e.*, at the dash in the sequence amino acid NLG-MIM. This is typical of a membrane protein.

Table 7B. Encoded GPCR7 protein sequence (SEQ ID NO:22).

MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMYFFLTNLAFVD
LCYTSNATPQMSTNIVSEKTISFAGCFTQCYIFIALLLTEFYMLAAMAYDRYVAIYDPLRYSVKTSRRVC
ICLATFPYVYGFSDGLFQAILTFRLTFCRSNVINHFYCADPPLIKLSCSDTYVKEHAMFISAGFNLSSSL
TIVLVSYAFILAAILRIKSAEGRHKAFSTCGSHMMAVTLFYGTLFCMYIRPPTDKTVEESKIIAVFYTFV
SPVLNPLIYSLRNKDVKQALKNVLR

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of GPCR7 has 633 of 959 bases (66%) identical to a gb:GENBANK-ID:GGCOR2GEN |acc:X94742.1 mRNA from *Gallus gallus* COR2 (SEQ ID NO:51) (Table. 7C).

Table 7C. BLASTN of GPCR7 against COR2

>gb:GENBANK-ID:GGCOR2GEN|acc:X94742.1 *G. gallus* cor2 DNA for olfactory receptor 2 - (SEQ ID NO:51) *Gallus gallus*, 996 bp.
Score = 1415 (212.3 bits), Expect = 5.9e-58, P = 5.9e-58
Identities = 633/959 (66%), Positives = 633/959 (66%), Strand = Plus / Plus

```
Query:     7 AAGAGCAAACTGTCAGGA-AT-ATGTCCAACACAAATGGCAGTGCAATCACAGAATTCAT 64
             ||  |||| ||||   |  || ||| ||||   ||||  |||  | ||||| |||||  |
Sbjct:    37 AACTGCAA-CTGTGTTGTGATGATGGCCAAGGGAAATCACAGCTCCATCACTGAATTTGT 95

Query:    65 TTTACTTGGGCT-CACAGATTGCCCGGAACTCCAGTCTCTGCTTTTTGTGCTGTTTCTGG 123
               | |||||| | | | ||      ||   ||||| || | || ||| ||  | |||| |
Sbjct:    96 GCT-CTTGGGATTCTCTGAAAAGAGGGCCATCCAGGCTGTTCTCTTTATGG-GCTTCTTG 153

Query:   124 TTGTT-TACCTCGTCACCCTGCTAGGCAACCTGGGCATGATAATGTTAATGAGACTGGAC 182
              || | |||||  |||| ||||||||||  |||||||||| |  || || || ||||||
Sbjct:   154 CTGATCTACCTGATCACTCTGCTAGGCAATGTGGGCATGATCACATTGATCAGGCTGGAC 213

Query:   183 TCTCGCCTTCACACGCCCATGTACTTCTTCCTCACTAACTTAGCCTTTGTGGATTTGTGC 242
             || || |||||||| || |||||||||||||| |  | |||  ||||  | ||| | |||
Sbjct:   214 TCCCGGCTTCACACCCCTATGTACTTCTTCCTGAGCAGCTTGTCCTTCCTCGATATCTGC 273

Query:   243 TATACATC-A-AATGCAACCC-CGCA-GATGTC-GACTAATATC-GTATCTGAGAAGACC 296
             ||| | || | ||| ||  || ||   | | || |||   |  | | |||  ||||   |
Sbjct:   274 TATTCCTCCACAAT-CACTCCTCGAGTGCTCTCAGACC--TCCCAGCATCACAGAAAGTC 330

Query:   297 ATTTCCTTTGCTGGTTGCTTTACACAGTGCTACATTTTCATTGCCCTT-CTACTCACTGA 355
             ||||||    |||  ||| |  |||||| || | | |  || | || | || ||| ||
Sbjct:   331 ATTTCCCACTCTGCATGCCTGGCACAGTTTTATTTCTACGCTGTCTTTGCCAC-CACAGA 389
```

```
Query:  356 GTTTTACATGCTGGCAGCAATGGCCTATGACCGCTATGTGGCCATATATGACCCTCTGCG 415
Sbjct:  390 GTGCTATCTTTTGGCCGCAATGGCATATGACCGCTACGTGGCCATCTGCAGCCCTCTGCT 449

Query:  416 CTACAGTGTGAAA-ACGTCCAGGAGAGTTTGCAT-CTGCTTG--GCCACATT-TCCCTAT 470
Sbjct:  450 CTAT-GTCTTCTCCATGTCCAGCAGAGTTTGTGTGCTGCTGGTTGCTGGCTCATACCT-T 507

Query:  471 GTCTATGGCTTCTCAGATGG-ACTCTTCCAGGCCATCCTGAC-CTTCCGCCTGACCTTCT 528
Sbjct:  508 GTCGG-GG-TTGTGA-ATGCCACCATTC-ACACAGGGCTTGCACTGCAGC-TGTCCTTCT 562

Query:  529 GTAGATCCAATGTCATCAACCACTTCTACTGTG-CTGACCCGCCGCTCATTAAGCTTTCT 587
Sbjct:  563 GTGGTCCCAACATCATCAATCACTTCTACTGTGACGGTCCC-CCGCTC-T-ACGCCATCT 619

Query:  588 TGTTCTGATACTT-ATGTCAAA-GAGCATGCCATGTTCATATCTGCT-GGCTTCAACCT- 643
Sbjct:  620 CGTGCACAGACCCCACCACCAACGAGATTGCGATATTTCT-TGTGGTTGGCTTCAACATG 678

Query:  644 CTCCAGCTCCCTCACCATCGTCTTGGTGTCCTATGCCTTCATTCTTGCT-GCCATCCTCC 702
Sbjct:  679 CTC-ATCACCAGCGTGACCATCTTCATCTCCTACACCTACATCCT-GTTCGCTGTCCTCA 736

Query:  703 GGAT-CAAATCAGCAG-AGGGAAGGCACAAGGCATTCTCCACCTGTGGTTCCCATATGAT 760
Sbjct:  737 GGATGCACA-CAGCTGCAGGCAAA-CGCAAAACCTTCTCCACGTGTGCGTCCCACCTGGC 794

Query:  761 GGCTGTCACCCTGTTTTATGGGACTCT-CTT-TTGCATGTATATAAGACC-ACCAACAGA 817
Sbjct:  795 CACCGTCACCCTATTCTATGC--CTCTGCTGGTTCCATGTACTCACGGCCCAGCTCCAGG 852

Query:  818 TAAGACT-GTTGAGGAATCTAAAATAATAGCTGTCTTTTACACCTTTGTGAGTCCGGTAC 876
Sbjct:  853 CACTCCCAGGACCTGGA-C-AAGGTGGCCTCTGTGTTCTACACCATGGTGACCCCCATGC 910

Query:  877 TTAATCCATTGATCTACAGTCTGAGGAATAAAGATGTGAAGCAGGCCTTGAAGAATGTCC 936
Sbjct:  911 TGAACCCCCTCATCTACAGCCTGAGGAACCAGGAGGTAAAGGATGTTTTAGGGAAAGTGA 970

Query:  937 TGAGATGAAATATTGTCA-TGACCA 960
Sbjct:  971 TGGGGAGGAAGAGTGTCTCTGACAA 995
```

The GPCR7 amino acid has 164 of 305 amino acid residues (53%) identical to, and 214 of 305 amino acid residues (70%) similar to, the 309 amino acid OR M72 (ptnr:TREMBLNEW-Acc No.:AAG09780) protein from *Mus musculus* OR M72, (SEQ ID NO:52) (Table 7D).

Table 7D. BLASTP alignments of GPCR7 against OR M72, (SEQ ID NO:52)

```
>ptnr:TREMBLNEW-ACC:AAG09780 ODORANT RECEPTOR M72 - Mus musculus (Mouse), 309 aa.
 Score = 811 (285.5 bits), Expect = 1.2e-80, P = 1.2e-80
 Identities = 164/305 (53%), Positives = 214/305 (70%)

Query:    1 MSNTNGSAITEFILLGLTDCPELQSLLFVLFLVVYLVTLLGNLGMIMLMRLDSRLHTPMY 60
Sbjct:    1 MAAENQSTVTEFILRGLTNRPELQLPLLLLFLGIYIVTMVGNLGMITLIGLNSQLHTPMY 60

Query:   61 FFLTNLAFVDLCYTSNATPQMSTNIVSEKT-ISFAGCFTQCYIFIALLLTEFYMLAAMAY 119
Sbjct:   61 FFLSNLSLVDLCYSSVITPKMLINFVSQRNLISYVGCMSQLYFFLVFVIAECYMLTVMAY 120
```

```
Query:   120 DRYVAIYDPLRYSVKTSRRVCICLATFPYVYGFSDGLFQAILTFRLTFCRSNVINHFYCA 179
             ||||||  || |++  |  +|  |  | |  |      +  |  +| +|  ++|+|++|
Sbjct:   121 DRYVAICQPLLYNIIMSPALCSLLVVFVYAMGLIGSTIETSLMLKLNYCE-DLISHYFCD 179

Query:   180 DPPLIKLSCSDTYVKEHAMFISAGFNLS-SSLTIVLVSYAFILAAILRIKSAEGRHKAFS 238
               ||+|||||  ||  | |+|  ||||+  +||| ||+|||||++|||| | ||| ||||
Sbjct:   180 ILPLMKLSCSSTYDIEMAVFFLAGFNIIVTSLT-VLISYAFILSSILRISSNEGRSKAFS 238

Query:   239 TCGSHMMAVTLFYGTLFCMYIRPPTDKTVEESKIIAVFYTFVSPVLNPLIYSLRNKDVKQ 298
             || ||  || ||||+   ||++| |  ++ +  + +|||| | |+ ||||||||||+||
Sbjct:   239 TCSSHFAAVGLFYGSTAFMYLKPSTASSLAQENVASVFYTTVIPMFNPLIYSLRNKEVKT 298

Query:   299 ALKNVLR 305
             ||  ||
Sbjct:   299 ALDKTLR 305
```

The presence of identifiable domains in GPCR7 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, and Prints followed by determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/interpro/). The results indicate that this protein contains the following protein domains (as defined by Interpro) at the indicated positions: domain name 7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family) at amino acid positions 41 to 289. This indicates that the sequence of GPCR7 has properties similar to those of other proteins known to contain this domain.

GPCR7 maps to chromosome 11. This information was assigned using the Online Mendelian Inheritance in Man (OMIM) database, the electronic northern bioinformatic tool implemented by CuraGen Corporation, public ESTs, public literature references and/or genomic clone homologies. This was executed to derive the chromosomal mapping of the Genomic clones. literature references and/or EST sequences that were included in the invention.

GPCR7 is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that

were included in the invention including but not limited to Public EST sources, Genomic Clone sources, Literature sources, and/or RACE sources.

The protein similarity information, expression pattern, and map location for the GPCR7 protein and nucleic acid suggest that GPCR7 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family. Therefore, GPCR7 are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon.

GPCR7 is useful in potential diagnostic and therapeutic applications implicated in various GPCR- or OR-related diseases and disorders described below and/or other pathologies. For example, the compositions of GPCR7 will have efficacy for treatment of patients suffering from: : Familial Mediterranian Fever, developmental diseases, MHCII and III diseases (immune diseases), Taste and scent detectability Disorders, Burkitt's lymphoma, Corticoneurogenic disease, Signal Transduction pathway disorders, Retinal diseases including those involving photoreception, Cell Growth rate disorders; Cell Shape disorders, Feeding disorders;control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of apetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Ostoeodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidoluysian atrophy(DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like. The polypeptides can be used as immunogens to produce antibodies specific for GPCR7, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the GPCR7-like protein may be useful in gene therapy, and

the GPCR7-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of GPCR7 will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Ostoeodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The GPCR7 nucleic acid and protein , or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to GPCR7 for use in therapeutic or diagnostic methods. Other GPCR-related diseases and disorders are contemplated.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR7 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. In one embodiment, a contemplated GPCR7 epitope is from aa 15 to 70. In another embodiment, a GPCR7 epitope is from aa 85 to 125. In additional embodiments, GPCR7 epitopes are from aa 140 to 175, from aa 210 to 235, from aa 240 to 260, and from aa 275 to 290.

A summary of the GPCRX nucleic acids and proteins of the invention is provided in Table 8A. A summary of homologous sequences identified in searches of available sequence databases is provided in Table 8B.

TABLE 8A: Summary Of Nucleic Acids And Proteins Of The Invention

Name	Tables	Clone; Description of Homolog	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO
GPCR1	1A, 1B, 1C	AL031943_A; CG54236-02; GPCR-like protein, cysteinyl leukotriene receptor-like protein	1	2
GPCR2	2A, 2B	AC022289_A; OR-like protein	3	4
	2C, 2D	AC022289_A1; OR-like protein	5	6

GPCR3	3A, 3B	AP001112_A; OR-like protein	7	8
GPCR4	4A, 4B	AP001112_B; OR-like protein	9	10
	4C, 4D	AC020597A; OR-like protein	11	12
GPCR5	5A, 5B	AP001112_C; OR-like protein	13	14
	5C, 5D	AC0170103B1; OR-like protein	15	16
	5E, 5F	CG50173-01; OR-like protein	17	18
GPCR6	6A, 6B	AP001112_D; OR-like protein	19	20
GPCR7	7A, 7B	AP001112_da1; OR-like protein	21	22
GPCR1	Example 3	Ag2695 Forward	63	
GPCR1	Example 3	Ag2695 Probe	64	
GPCR1	Example 3	Ag2695 Reverse	65	

TABLE 8B: Summary of Query Sequences Disclosed

Table	Database	Acc. No.	Sequence Name	Species	SEQ ID NO.
1D	GenBank	L06109	activated T cell-specific G PCR mRNA	chicken	23
1E	GenBank	XM_007164.1	cysteinyl leukotriene CysLT2 receptor	human	24
1F	trEmblnew	CAA73144	P2Y-Like G-Protein Coupled Receptor	human	25
1G, 1H	GenBank	XP_007164	translation, cysteinyl leukotriene CysLT2 receptor	human	26
1H	Patp	W75799	unknown	human	27
1H	strpEmbl	P34996	P2Y Purinoceptor 1	chicken	28
1I, 2J, 3H, 4O, 5M, 6K	Pfam	7tm_1	7 transmembrane receptor (rhodopsin family) fragment ; residues 1-180	consensus	29
2E	GenBank	450948	TB 567	rat	30
2F	GenBank	AFD65860	ORD 3; residues 437-644	human	31
2F	GenBank	AFD65860	ORD-3; residues 121-219	human	32
2G, 4N, 5L, 6J	SwissProt	Q13606	OLF-1	human	33
2H, 4M	strpEmbl	CAA64370	OR	chicken	34
2I	SWISS	P37070	OLF	chicken	35
2I	GenBank	Q63395	OLF	rat	36
2J, 4O, 5M, 6K	Pfam	7tm-1	7 transmembrane receptor (rhodopsin family) fragment; residues 310-377	consensus	37
3C	GenBank	AF045577	OR93	chimp	38
3D	GenBank	NM_013728	ORfr 4-3; residues 835-907	mouse	39
3D	GenBank	NM_013728	ORfr 4-3; residues 163-210	mouse	40
3E, 3G, 4I, 4N	sptrEmbl	O77756	OLF; residues 5-309	chimp	41
3F, 3G, 4N	GenBank	AF20365	OLF	mouse	42
3G, 4J, 4N, 5H, 5L, 6J	sptrEmbl	O77758	OLF	gibbon	43
4E	GenBank	U50948	TB 567	rat	44

4F	GenBank	AF247656	M72; residues 821-890	mouse	45
4F	GenBank	AF247656	M72; residues 160-201	mouse	46
4G	GenBank	AF282291	OR K42	mouse	47
4H	GenBank	AF282298	OR K40	mouse	48
4K	GenBank	AF282291	OR K42	mouse	49
4L	GenBank	NP_006628	OR 5I1	human	50
5G, 6C	GenBank	X94742	COR2	chicken	51
5I	GenBank	AAG09870	M72	mouse	52
5J	GenBank	AAG39876	OR K42	mouse	53
5K	GenBank	AAG29379	M71	mouse	54
5L	sptrEmbl	Q63394	OLF	rat	55
6D, 6J	sptrEmbl	Q90808	OR4	chicken	56
6E	GenBank	AAC63969	OR93Ch	chimp	57
6F	GenBank	AAC63971	OR93Gib	gibbon	58
6G	EMBL	CAA64368	COR2	chicken	59
6H	GenBank	AAG39871	K30	mouse	60
6I	GenBank	AAG39856	OR K11	mouse	61
6J	GenBank	NP_068632	OR G264617	rat	62

GPCRX Nucleic Acids and Polypeptides

One aspect of the invention pertains to isolated nucleic acid molecules that encode GPCRX polypeptides or biologically-active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify GPCRX-encoding nucleic acids (*e.g.*, GPCRX mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of GPCRX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An GPCRX nucleic acid can encode a mature GPCRX polypeptide. As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an ORF described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in

which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an ORF, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term "probes", as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated GPCRX nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 as a hybridization probe, GPCRX molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to GPCRX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or a portion of this nucleotide sequence (*e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an GPCRX polypeptide). A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9,

11, 13, 15, 17, 19 and 21, that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under

stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of GPCRX polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for an GPCRX polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human GPCRX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, as well as a polypeptide possessing GPCRX biological activity. Various biological activities of the GPCRX proteins are described below.

An GPCRX polypeptide is encoded by the open reading frame ("ORF") of an GPCRX nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human GPCRX genes allows for the generation of probes and primers designed for use in identifying and/or cloning GPCRX homologues in other cell types, *e.g.* from other tissues, as well as GPCRX homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7,

9, 11, 13, 15, 17, 19 and 21; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21.

Probes based on the human GPCRX nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an GPCRX protein, such as by measuring a level of an GPCRX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting GPCRX mRNA levels or determining whether a genomic GPCRX gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an GPCRX polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of GPCRX" can be prepared by isolating a portion of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, that encodes a polypeptide having an GPCRX biological activity (the biological activities of the GPCRX proteins are described below), expressing the encoded portion of GPCRX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of GPCRX.

GPCRX Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, due to degeneracy of the genetic code and thus encode the same GPCRX proteins as that encoded by the nucleotide sequences shown in SEQ ID NO NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22.

In addition to the human GPCRX nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the GPCRX polypeptides may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the GPCRX genes may exist among individuals within a population due to

natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an GPCRX protein, preferably a vertebrate GPCRX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the GPCRX genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the GPCRX polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the GPCRX polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding GPCRX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the GPCRX cDNAs of the invention can be isolated based on their homology to the human GPCRX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding GPCRX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The

Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, *e.g.*, Ausubel, et *al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* 78: 6789-6792.

Conservative Mutations

In addition to naturally-occurring allelic variants of GPCRX sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, thereby leading to changes in the amino acid sequences of the encoded GPCRX proteins, without altering the functional ability of said GPCRX proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the GPCRX proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the GPCRX proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding GPCRX proteins that contain changes in amino acid residues that are not essential for activity. Such GPCRX proteins differ in amino acid sequence from SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14,

16, 18, 20 and 22; more preferably at least about 70% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22; still more preferably at least about 80% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22; even more preferably at least about 90% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22; and most preferably at least about 95% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22.

An isolated nucleic acid molecule encoding an GPCRX protein homologous to the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the GPCRX protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an GPCRX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for GPCRX biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be

substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant GPCRX protein can be assayed for (*i*) the ability to form protein:protein interactions with other GPCRX proteins, other cell-surface proteins, or biologically-active portions thereof, (*ii*) complex formation between a mutant GPCRX protein and an GPCRX ligand; or (*iii*) the ability of a mutant GPCRX protein to bind to an intracellular target protein or biologically-active portion thereof; (*e.g.* avidin proteins).

In yet another embodiment, a mutant GPCRX protein can be assayed for the ability to regulate a specific biological function (*e.g.*, regulation of insulin release).

Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (*e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire GPCRX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an GPCRX protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22; or antisense nucleic acids complementary to an GPCRX nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an GPCRX protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the GPCRX protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the GPCRX protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of GPCRX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of GPCRX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of GPCRX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an GPCRX protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional

nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (*e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other. See, *e.g.*, Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, *e.g.*, Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a chimeric RNA-DNA analogue (see, *e.g.*, Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330.

Ribozymes and PNA Moieties

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave GPCRX mRNA transcripts to thereby inhibit translation of GPCRX mRNA. A ribozyme having specificity for an GPCRX-encoding nucleic acid can be designed based upon the nucleotide sequence of an GPCRX cDNA disclosed herein (*i.e.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in

which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an GPCRX-encoding mRNA. See, *e.g.*, U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* GPCRX mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, GPCRX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the GPCRX nucleic acid (*e.g.*, the GPCRX promoter and/or enhancers) to form triple helical structures that prevent transcription of the GPCRX gene in target cells. *See, e.g.,* Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the GPCRX nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. *See, e.g.,* Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (*e.g.*, DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

PNAs of GPCRX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of GPCRX can also be used, for example, in the analysis of single base pair mutations in a gene (*e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S_1 nucleases (*see,* Hyrup, *et al.*, 1996. *supra*); or as probes or primers for DNA sequence and hybridization (*see,* Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNAs of GPCRX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of GPCRX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA

recognition enzymes (*e.g.*, RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see*, Hyrup, etal., 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.*, 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.*, Mag, *et al.*, 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.*, Finn, *et al.*, 1996. *supra*. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.*, Petersen, *et al.*, 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; Lemaitre, *et al.*, 1987. *Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g.*, Krol, *et al.*, 1988. *BioTechniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon, 1988. *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

GPCRX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of GPCRX polypeptides whose sequences are provided in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, while still encoding a protein that maintains its GPCRX activities and physiological functions, or a functional fragment thereof.

In general, an GPCRX variant that preserves GPCRX-like function includes any variant in which residues at a particular position in the sequence have been substituted by

other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated GPCRX proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-GPCRX antibodies. In one embodiment, native GPCRX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, GPCRX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an GPCRX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the GPCRX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of GPCRX proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes preparations of GPCRX proteins having less than about 30% (by dry weight) of non-GPCRX proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-GPCRX proteins, still more preferably less than about 10% of non-GPCRX proteins, and most preferably less than about 5% of non-GPCRX proteins. When the GPCRX protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the GPCRX protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of GPCRX proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of GPCRX proteins having less than about 30% (by dry weight) of chemical precursors or non-GPCRX chemicals, more preferably less than about 20% chemical precursors or

non-GPCRX chemicals, still more preferably less than about 10% chemical precursors or non-GPCRX chemicals, and most preferably less than about 5% chemical precursors or non-GPCRX chemicals.

Biologically-active portions of GPCRX proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the GPCRX proteins (*e.g.*, the amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22) that include fewer amino acids than the full-length GPCRX proteins, and exhibit at least one activity of an GPCRX protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the GPCRX protein. A biologically-active portion of an GPCRX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native GPCRX protein.

In an embodiment, the GPCRX protein has an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22. In other embodiments, the GPCRX protein is substantially homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, and retains the functional activity of the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the GPCRX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, and retains the functional activity of the GPCRX proteins of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22.

Determining Homology Between Two or More Sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See,* Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

Chimeric and Fusion Proteins

The invention also provides GPCRX chimeric or fusion proteins. As used herein, an GPCRX "chimeric protein" or "fusion protein" comprises an GPCRX polypeptide operatively-linked to a non-GPCRX polypeptide. An "GPCRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an GPCRX protein (SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22), whereas a "non-GPCRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the GPCRX protein, *e.g.*, a protein that is different from the GPCRX protein and that is derived from the same or a different organism. Within an GPCRX fusion protein the GPCRX polypeptide can correspond to all or a portion of an GPCRX protein. In one embodiment, an GPCRX fusion protein comprises at least one biologically-active portion of an GPCRX protein. In another embodiment, an GPCRX fusion protein comprises at least two

biologically-active portions of an GPCRX protein. In yet another embodiment, an GPCRX fusion protein comprises at least three biologically-active portions of an GPCRX protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the GPCRX polypeptide and the non-GPCRX polypeptide are fused in-frame with one another. The non-GPCRX polypeptide can be fused to the N-terminus or C-terminus of the GPCRX polypeptide.

In one embodiment, the fusion protein is a GST-GPCRX fusion protein in which the GPCRX sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant GPCRX polypeptides.

In another embodiment, the fusion protein is an GPCRX protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of GPCRX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an GPCRX-immunoglobulin fusion protein in which the GPCRX sequences are fused to sequences derived from a member of the immunoglobulin protein family. The GPCRX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an GPCRX ligand and an GPCRX protein on the surface of a cell, to thereby suppress GPCRX-mediated signal transduction *in vivo*. The GPCRX-immunoglobulin fusion proteins can be used to affect the bioavailability of an GPCRX cognate ligand. Inhibition of the GPCRX ligand/GPCRX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the GPCRX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-GPCRX antibodies in a subject, to purify GPCRX ligands, and in screening assays to identify molecules that inhibit the interaction of GPCRX with an GPCRX ligand.

An GPCRX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including

automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.*, Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An GPCRX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the GPCRX protein.

GPCRX Agonists and Antagonists

The invention also pertains to variants of the GPCRX proteins that function as either GPCRX agonists (*i.e.*, mimetics) or as GPCRX antagonists. Variants of the GPCRX protein can be generated by mutagenesis (*e.g.*, discrete point mutation or truncation of the GPCRX protein). An agonist of the GPCRX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the GPCRX protein. An antagonist of the GPCRX protein can inhibit one or more of the activities of the naturally occurring form of the GPCRX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the GPCRX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the GPCRX proteins.

Variants of the GPCRX proteins that function as either GPCRX agonists (*i.e.*, mimetics) or as GPCRX antagonists can be identified by screening combinatorial libraries of mutants (*e.g.*, truncation mutants) of the GPCRX proteins for GPCRX protein agonist or antagonist activity. In one embodiment, a variegated library of GPCRX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of GPCRX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential GPCRX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of GPCRX sequences therein. There are a variety of methods which can be used to produce libraries of potential GPCRX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate

set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential GPCRX sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. *See, e.g.,* Narang, 1983. *Tetrahedron* 39: 3; Itakura, *et al.*, 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, *et al.*, 1984. *Science* 198: 1056; Ike, *et al.*, 1983. *Nucl. Acids Res.* 11: 477.

Polypeptide Libraries

In addition, libraries of fragments of the GPCRX protein coding sequences can be used to generate a variegated population of GPCRX fragments for screening and subsequent selection of variants of an GPCRX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an GPCRX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S_1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the GPCRX proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of GPCRX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify GPCRX variants. *See, e.g.,* Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, *et al.*, 1993. *Protein Engineering* 6:327-331.

Anti-GPCRX Antibodies

The invention encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the GPCRX polypeptides of said invention.

An isolated GPCRX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind to GPCRX polypeptides using standard techniques for polyclonal and monoclonal antibody preparation. The full-length GPCRX proteins can be used or, alternatively, the invention provides antigenic peptide fragments of GPCRX proteins for use as immunogens. The antigenic GPCRX peptides comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, and encompasses an epitope of GPCRX such that an antibody raised against the peptide forms a specific immune complex with GPCRX. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of GPCRX that is located on the surface of the protein (*e.g.*, a hydrophilic region). As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation (*see, e.g.*, Hopp and Woods, 1981. *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle, 1982. *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety).

As disclosed herein, GPCRX protein sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically-binds (immunoreacts with) an antigen, such as GPCRX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human GPCRX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an GPCRX protein sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic

preparation can contain, for example, recombinantly-expressed GPCRX protein or a chemically-synthesized GPCRX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille-Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against GPCRX can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of GPCRX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular GPCRX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular GPCRX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (*see, e.g.*, Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*see, e.g.*, Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the invention and may be produced by using human hybridomas (*see, e.g.*, Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an GPCRX protein (*see, e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (*see, e.g.*, Huse, *et al.*, 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for an GPCRX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. *See, e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to an GPCRX protein may be produced by techniques known in the

art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent; and (*iv*) F_v fragments.

Additionally, recombinant anti-GPCRX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314 :446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi, *et al.* (1986) *BioTechniques* 4:214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an GPCRX protein is facilitated by generation of hybridomas that bind to the fragment of an GPCRX protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an GPCRX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-GPCRX antibodies may be used in methods known within the art relating to the localization and/or quantitation of an GPCRX protein (*e.g.*, for use in measuring levels of the GPCRX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for GPCRX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody

derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-GPCRX antibody (*e.g.*, monoclonal antibody) can be used to isolate an GPCRX polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-GPCRX antibody can facilitate the purification of natural GPCRX polypeptide from cells and of recombinantly-produced GPCRX polypeptide expressed in host cells. Moreover, an anti-GPCRX antibody can be used to detect GPCRX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the GPCRX protein. Anti-GPCRX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

GPCRX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an GPCRX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, GPCRX proteins, mutant forms of GPCRX proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of GPCRX proteins in prokaryotic or eukaryotic cells. For example, GPCRX proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San

Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (*i*) to increase expression of recombinant protein; (*ii*) to increase the solubility of the recombinant protein; and (*iii*) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.*, Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the GPCRX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerivisae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, GPCRX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the α-fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to GPCRX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA

molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, GPCRX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a

selectable marker can be introduced into a host cell on the same vector as that encoding GPCRX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) GPCRX protein. Accordingly, the invention further provides methods for producing GPCRX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding GPCRX protein has been introduced) in a suitable medium such that GPCRX protein is produced. In another embodiment, the method further comprises isolating GPCRX protein from the medium or the host cell.

Transgenic GPCRX Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which GPCRX protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous GPCRX sequences have been introduced into their genome or homologous recombinant animals in which endogenous GPCRX sequences have been altered. Such animals are useful for studying the function and/or activity of GPCRX protein and for identifying and/or evaluating modulators of GPCRX protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous GPCRX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing GPCRX-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral

infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human GPCRX cDNA sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human GPCRX gene, such as a mouse GPCRX gene, can be isolated based on hybridization to the human GPCRX cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the GPCRX transgene to direct expression of GPCRX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the GPCRX transgene in its genome and/or expression of GPCRX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding GPCRX protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an GPCRX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the GPCRX gene. The GPCRX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21), but more preferably, is a non-human homologue of a human GPCRX gene. For example, a mouse homologue of human GPCRX gene of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, can be used to construct a homologous recombination vector suitable for altering an endogenous GPCRX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous GPCRX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous GPCRX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous GPCRX protein). In the homologous recombination vector, the altered

portion of the GPCRX gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the GPCRX gene to allow for homologous recombination to occur between the exogenous GPCRX gene carried by the vector and an endogenous GPCRX gene in an embryonic stem cell. The additional flanking GPCRX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.,* Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is ten introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced GPCRX gene has homologously-recombined with the endogenous GPCRX gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*. *See,* O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.*, a somatic cell) from the transgenic animal can be isolated and induced to exit the

growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g.*, the somatic cell) is isolated.

Pharmaceutical Compositions

The GPCRX nucleic acid molecules, GPCRX proteins, and anti-GPCRX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be

adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an GPCRX protein or anti-GPCRX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form

of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express GPCRX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect GPCRX mRNA (*e.g.*, in a biological sample) or a genetic lesion in an GPCRX gene, and to modulate GPCRX activity, as described further, below. In addition, the GPCRX proteins can be used to screen drugs or compounds that modulate the GPCRX protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of GPCRX protein or production of GPCRX protein forms that have decreased or aberrant activity compared to GPCRX wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease(possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-GPCRX antibodies of the invention can be used to

detect and isolate GPCRX proteins and modulate GPCRX activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to GPCRX proteins or have a stimulatory or inhibitory effect on, *e.g.*, GPCRX protein expression or GPCRX protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an GPCRX protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of GPCRX protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an GPCRX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the GPCRX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the GPCRX protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of GPCRX protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds GPCRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCRX protein, wherein determining the ability of the test compound to interact with an GPCRX protein comprises determining the ability of the test compound to preferentially bind to GPCRX protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of GPCRX protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the GPCRX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCRX or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the GPCRX protein to bind to or interact with an

GPCRX target molecule. As used herein, a "target molecule" is a molecule with which an GPCRX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an GPCRX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An GPCRX target molecule can be a non-GPCRX molecule or an GPCRX protein or polypeptide of the invention. In one embodiment, an GPCRX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound GPCRX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with GPCRX.

Determining the ability of the GPCRX protein to bind to or interact with an GPCRX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the GPCRX protein to bind to or interact with an GPCRX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an GPCRX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an GPCRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the GPCRX protein or biologically-active portion thereof. Binding of the test compound to the GPCRX protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the GPCRX protein or biologically-active portion thereof with a known compound which binds GPCRX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCRX protein, wherein determining the ability of the test compound to interact with an GPCRX protein comprises determining the ability of the test compound to preferentially bind to GPCRX or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting GPCRX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the GPCRX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCRX can be accomplished, for example, by determining the ability of the GPCRX protein to bind to an GPCRX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of GPCRX protein can be accomplished by determining the ability of the GPCRX protein further modulate an GPCRX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the GPCRX protein or biologically-active portion thereof with a known compound which binds GPCRX protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCRX protein, wherein determining the ability of the test compound to interact with an GPCRX protein comprises determining the ability of the GPCRX protein to preferentially bind to or modulate the activity of an GPCRX target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of GPCRX protein. In the case of cell-free assays comprising the membrane-bound form of GPCRX protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of GPCRX protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either GPCRX protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to GPCRX protein, or interaction of GPCRX protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such

vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-GPCRX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or GPCRX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of GPCRX protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the GPCRX protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated GPCRX protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with GPCRX protein or target molecules, but which do not interfere with binding of the GPCRX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or GPCRX protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the GPCRX protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the GPCRX protein or target molecule.

In another embodiment, modulators of GPCRX protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of GPCRX mRNA or protein in the cell is determined. The level of expression of GPCRX mRNA or protein in the presence of the candidate compound is compared to the level of expression of GPCRX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of GPCRX mRNA or protein expression based upon this comparison. For example, when expression of GPCRX mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than

in its absence, the candidate compound is identified as a stimulator of GPCRX mRNA or protein expression. Alternatively, when expression of GPCRX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of GPCRX mRNA or protein expression. The level of GPCRX mRNA or protein expression in the cells can be determined by methods described herein for detecting GPCRX mRNA or protein.

In yet another aspect of the invention, the GPCRX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with GPCRX ("GPCRX-binding proteins" or "GPCRX-bp") and modulate GPCRX activity. Such GPCRX-binding proteins are also likely to be involved in the propagation of signals by the GPCRX proteins as, for example, upstream or downstream elements of the GPCRX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for GPCRX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an GPCRX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with GPCRX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (*i*) map their respective

genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (*ii*) identify an individual from a minute biological sample (tissue typing); and (*iii*) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the GPCRX sequences, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or fragments or derivatives thereof, can be used to map the location of the GPCRX genes, respectively, on a chromosome. The mapping of the GPCRX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, GPCRX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the GPCRX sequences. Computer analysis of the GPCRX, sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the GPCRX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. *See, e.g.*, D'Eustachio, *et al.*, 1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using

a single thermal cycler. Using the GPCRX sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see*, Verma, *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.*, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the GPCRX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete

sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

The GPCRX sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the GPCRX sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The GPCRX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for

prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining GPCRX protein and/or nucleic acid expression as well as GPCRX activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant GPCRX expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with GPCRX protein, nucleic acid expression or activity. For example, mutations in an GPCRX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with GPCRX protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining GPCRX protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCRX in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of GPCRX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting GPCRX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes GPCRX protein such that the presence of GPCRX is detected in the biological sample. An agent for detecting GPCRX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to GPCRX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length GPCRX nucleic acid, such as the

nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to GPCRX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting GPCRX protein is an antibody capable of binding to GPCRX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect GPCRX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of GPCRX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of GPCRX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of GPCRX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of GPCRX protein include introducing into a subject a labeled anti-GPCRX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting GPCRX protein, mRNA, or genomic DNA, such that the presence of GPCRX protein, mRNA or genomic DNA is detected in the biological sample, and comparing

the presence of GPCRX protein, mRNA or genomic DNA in the control sample with the presence of GPCRX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of GPCRX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting GPCRX protein or mRNA in a biological sample; means for determining the amount of GPCRX in the sample; and means for comparing the amount of GPCRX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect GPCRX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant GPCRX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with GPCRX protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant GPCRX expression or activity in which a test sample is obtained from a subject and GPCRX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of GPCRX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant GPCRX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant GPCRX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant GPCRX expression or activity in which a test sample is obtained and GPCRX protein or nucleic acid is detected (*e.g.*, wherein the presence of GPCRX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant GPCRX expression or activity).

The methods of the invention can also be used to detect genetic lesions in an GPCRX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an GPCRX-protein, or the misexpression of the GPCRX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (*i*) a deletion of one or more nucleotides from an GPCRX gene; (*ii*) an addition of one or more nucleotides to an GPCRX gene; (*iii*) a substitution of one or more nucleotides of an GPCRX gene, (*iv*) a chromosomal rearrangement of an GPCRX gene; (*v*) an alteration in the level of a messenger RNA transcript of an GPCRX gene, (*vi*) aberrant modification of an GPCRX gene, such as of the methylation pattern of the genomic DNA, (*vii*) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an GPCRX gene, (*viii*) a non-wild-type level of an GPCRX protein, (*ix*) allelic loss of an GPCRX gene, and (*x*) inappropriate post-translational modification of an GPCRX protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an GPCRX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the GPCRX-gene (*see,* Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an GPCRX gene under conditions such that hybridization and amplification of the GPCRX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see*, Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see*, Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Qβ Replicase (*see*, Lizardi, *et al.*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an GPCRX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.*, U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in GPCRX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. *See, e.g.*, Cronin, *et al.*, 1996. *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in GPCRX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al.*, *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the GPCRX gene and detect mutations by comparing the sequence of the sample GPCRX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures

can be utilized when performing the diagnostic assays (*see, e.g.*, Naeve, *et al.*, 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the GPCRX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.*, Myers, *et al.*, 1985. *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type GPCRX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S_1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.*, Cotton, *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.*, 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in GPCRX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. *See, e.g.*, Hsu, *et al.*, 1994. *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an GPCRX sequence, *e.g.*, a wild-type GPCRX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.*, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in GPCRX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g.*, Orita, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA*: 86: 2766; Cotton,

1993. *Mutat. Res.* 285: 125-144; Hayashi, 1992. *Genet. Anal. Tech. Appl.* 9: 73-79. Single-stranded DNA fragments of sample and control GPCRX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g.,* Keen, *et al.*, 1991. *Trends Genet.* 7: 5.

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g.,* Myers, *et al.*, 1985. *Nature* 313: 495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g.,* Rosenbaum and Reissner, 1987. *Biophys. Chem.* 265: 12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g.,* Saiki, *et al.*, 1986. *Nature* 324: 163; Saiki, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g.,* Gibbs, *et al.*, 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.,* Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel

restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.*, Gasparini, *et al.*, 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g.*, Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189. In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an GPCRX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which GPCRX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on GPCRX activity (*e.g.*, GPCRX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of GPCRX protein, expression of GPCRX nucleic acid, or mutation content of GPCRX genes in an

individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of GPCRX protein, expression of GPCRX nucleic acid, or mutation content of GPCRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness

phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an GPCRX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCRX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase GPCRX gene expression, protein levels, or upregulate GPCRX activity, can be monitored in clinical trails of subjects exhibiting decreased GPCRX gene expression, protein levels, or downregulated GPCRX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease GPCRX gene expression, protein levels, or downregulate GPCRX activity, can be monitored in clinical trails of subjects exhibiting increased GPCRX gene expression, protein levels, or upregulated GPCRX activity. In such clinical trials, the expression or activity of GPCRX and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including GPCRX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates GPCRX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of GPCRX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of GPCRX or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration

sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of an GPCRX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the GPCRX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the GPCRX protein, mRNA, or genomic DNA in the pre-administration sample with the GPCRX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of GPCRX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of GPCRX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant GPCRX expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Ostoeodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (*i*) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to an aforementioned peptide; (*iii*) nucleic acids encoding an aforementioned peptide; (*iv*) administration of antisense nucleic acid

and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endoggenous function of an aforementioned peptide by homologous recombination (*see*, *e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in-vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant GPCRX expression or activity, by administering to the subject an agent that modulates GPCRX expression or at least one GPCRX activity. Subjects at risk for a disease that is caused or contributed to by aberrant GPCRX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the GPCRX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of GPCRX aberrancy, for example, an GPCRX agonist or GPCRX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating GPCRX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of GPCRX protein activity associated with the cell. An agent that modulates GPCRX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an GPCRX protein, a peptide, an GPCRX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more GPCRX protein activity. Examples of such stimulatory agents include active GPCRX protein and a nucleic acid molecule encoding GPCRX that has been introduced into the cell. In another embodiment, the agent inhibits one or more GPCRX protein activity. Examples of such inhibitory agents include antisense GPCRX nucleic acid molecules and anti-GPCRX antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an GPCRX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, up-regulates or down-regulates) GPCRX expression or activity. In another embodiment, the method involves administering an GPCRX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant GPCRX expression or activity.

Stimulation of GPCRX activity is desirable in situations in which GPCRX is abnormally downregulated and/or in which increased GPCRX activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (*e.g.*, cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (*e.g.*, preclampsia).

Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts

the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Prophylactic and Therapeutic Uses of the Compositions of the Invention

The GPCRX nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the GPCRX protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the GPCRX protein, and the GPCRX protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

EXAMPLES

The following examples illustrate by way of non-limiting example various aspects of the invention.

The following examples illustrate by way of non-limiting example various aspects of the invention.

Example 1: Method of Identifying the Nucleic Acids

The novel nucleic acids of the invention were identified by TblastN using a proprietary sequence file, run against the Genomic Daily Files made available by GenBank. The nucleic acids were further predicted by the proprietary software program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length proteins.

Example 2. Quantitative expression analysis of GPCR2 in various cells and tissues

The quantitative expression of clone GPCR1 was assessed in a large number of normal and tumor sample cells and cell lines (Panel 1), as well as in surgical tissue samples (Panel 2), by real time quantitative PCR (TaqMan®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System.

First, 96 RNA samples were normalized to β-actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TaqMan® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; Catalog No. N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 ul and incubated for 30 min. at 48°C. cDNA (5 ul) was then transferred to a separate plate for the TaqMan® reaction using β-actin and GAPDH TaqMan® Assay Reagents (PE Biosystems; Catalog Nos. 4310881E and 4310884E, respectively) and TaqMan® universal PCR Master Mix (PE Biosystems; Catalog No. 4304447) according to the manufacturer's protocol. Reactions were performed in 25 ul using the following parameters: 2 min. at 50°C; 10 min. at 95°C; 15 sec. at 95°C/1 min. at 60°C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for ß-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their β-actin /GAPDH average CT values.

Normalized RNA (5 ul) was converted to cDNA and analyzed via TaqMan® using One Step RT-PCR Master Mix Reagents (PE Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software

package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (T_m) range = 58°-60° C, primer optimal Tm = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe T_m must be 10° C greater than primer T_m, amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl2, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/μl RNase inhibitor, and 0.25 U/μl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

The results for various cells and cell lines that constitute Panel 1 are shown in Table 10. In Table 10, the following abbreviations are used: ca. = carcinoma; * = established from metastasis; met = metastasis; s cell var= small cell variant; non-s = non-sm =non-small; squam = squamous; pl. eff = pl effusion = pleural effusion; glio = glioma; astro = astrocytoma; and neuro = neuroblastoma.

Panel 2 consists of a 96 well plate (2 control wells, 94 test samples) composed of RNA/cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues procured are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins". The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologists at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue,

in Tables 10 and 11). In addition, RNA/cDNA was obtained from various human tissues derived from human autopsies performed on deceased elderly people or sudden death victims (accidents, etc.). These tissue were ascertained to be free of disease and were purchased from various high quality commercial sources such as Clontech, Research Genetics, and Invitrogen.

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electrophoresis using 28s and 18s ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the presence of low molecular weight RNAs indicative of degradation products. Samples are quality controlled for genomic DNA contamination by reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

Example 3. Quantitative expression analysis of GPCR1 in various cells and tissues.

The quantitative expression of GPCR1 was assessed in a large number of normal and tumor sample cells and cell lines (Panel 1), as well as in surgical tissue samples (Panel 2), by real time quantitative PCR (TaqMan®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System as described above in Example 2, with the following primers (Table 9).

Table 9. Probe set Ag2695.

Primers	Sequences	T_m	Length	Start Pos.	SEQ ID NO:
Forward	5'-GGGAAATGGGTTGTCCATATAT-3'	58.8	22	266	63
Probe	FAM-5'-TCCTGCAGCCTTATAAGAAGTCCACA-3'-TAMRA	66.2	26	292	64
Reverse	5'-ATCTGAAATGGCCAGATTTAGC-3'	59.6	22	335	65

The TaqMan results for panels 1 and 2 are shown in Table 10.

Table 10. TaqMan results for GPCR1

Panel 1			Panel 2		
Tissue_Name	Run 1 Rel Expr. %	Run 2 Rel. Expr. %	Tissue_Name	Run 1 Rel. Expr. %	Run 2 Rel. Expr. %
Liver adenocarcinoma	0	0	Normal Colon GENPAK 061003	3.82	3.28
Pancreas	2.15	4.21	83220 CC NAT (ODO3866)	2.74	0.51
Pancreatic ca. CAPAN 2	0	0	83221 CC Gr.2 rectosigmoid (ODO3868)	0.13	0.21
Adrenal gland	67.36	100	83222 CC NAT (ODO3868)	0.92	0.27
Thyroid	5.08	1.96	83235 CC Mod Diff (ODO3920)	0.65	0.22
Salivary gland	5.11	3.54	83236 CC NAT (ODO3920)	0.46	0.28

Pituitary gland	1.05	4.9	83237 CC Gr.2 ascend colon (ODO3921)	3.21	2.24
Brain (fetal)	3.49	0	83238 CC NAT (ODO3921)	0.81	0.59
Brain (whole)	20.31	16.04	83241 CC from Partial Hepatectomy (ODO4309)	1.58	2.01
Brain (amygdala)	22.22	12.5	83242 Liver NAT (ODO4309)	1.26	1.47
Brain (cerebellum)	2.15	4.54	87472 Colon mets to lung (OD04451-01)	0.3	1.23
Brain (hippocampus)	61.13	35.11	87473 Lung NAT (OD04451-02)	2.88	1.71
Brain (thalamus)	8.72	18.56	Normal Prostate Clontech A+ 6546-1	2.01	0.77
Cerebral Cortex	45.06	53.22	84140 Prostate Cancer (OD04410)	1.88	1.72
Spinal cord	11.34	7.97	84141 Prostate NAT (OD04410)	3.52	3.9
CNS ca. (glio/astro)U87-MG	0	0	87073 Prostate Cancer (OD04720-01)	1.73	0.5
CNS ca. (glio/astro)U-118-MG	4.21	0	87074 Prostate NAT (OD04720-02)	5.01	4.42
CNS ca. (astro)SW1783	0	0	Normal Lung GENPAK 061010	6.93	7.8
CNS ca.* (neuro; met) SK-N-AS	0	0	83239 Lung Met to Muscle (ODO4286)	2.16	1.98
CNS ca. (astro) SF-539	8.96	0	83240 Muscle NAT (ODO4286)	0.3	0.51
CNS ca. (astro) SNB-75	0	0	84136 Lung Malignant Cancer (OD03126)	2.4	3.69
CNS ca. (glio) SNB-19	0	2.86	84137 Lung NAT (OD03126)	6.93	6.7
CNS ca. (glio) U251	0	0	84871 Lung Cancer (OD04404)	5.48	2.12
CNS ca. (glio) SF-295	0	1.96	84872 Lung NAT (OD04404)	1.55	1.91
Heart	41.47	32.99	84875 Lung Cancer (OD04565)	0.59	0.45
Skeletal muscle	0	0	85950 Lung Cancer (OD04237-01)	4.54	3.77
Bone marrow	3.49	11.74	85970 Lung NAT (OD04237-02)	3.93	5.18
Thymus	1.44	6.79	83255 Ocular Mel Met to Liver (ODO4310)	0	0.16
Spleen	100	59.05	83256 Liver NAT (ODO4310)	1.72	0.69
Lymph node	32.31	26.79	84139 Melanoma Mets to Lung (OD04321)	100	100
Colorectal	17.92	20.17	84138 Lung NAT (OD04321)	7.33	6.52
Stomach	9.54	0	Normal Kidney GENPAK 061008	6.38	5.04
Small intestine	13.4	38.96	83786 Kidney Ca, Nuclear grade 2 (OD04338)	34.15	33.92
Colon ca. SW480	0	0	83787 Kidney NAT (OD04338)	5.18	5.63
Colon ca.* (SW480 met)SW620	0	0	83788 Kidney Ca Nuclear grade 1/2 (OD04339)	1.39	0.78
Colon ca. HT29	0	1.9	83789 Kidney NAT (OD04339)	0.95	2.02
Colon ca.HCT-116	0	0	83790 Kidney Ca, Clear cell type (OD04340)	8.54	10.29
Colon ca. CaCo-2	0	0	83791 Kidney NAT (OD04340)	11.34	6.56
83219 CC Well to Mod Diff (ODO3866)	10.96	3.37	83792 Kidney Ca, Nuclear grade 3 (OD04348)	1.44	1.94
Colon ca. HCC-2998	0.62	0	83793 Kidney NAT (OD04348)	5.37	7.33
Gastric ca.* (liver met) NCI-N87	0	1.9	87474 Kidney Cancer (OD04622-01)	48.97	63.73
Bladder	0	2.42	87475 Kidney NAT (OD04622-03)	0.73	1.16
Trachea	4.15	0	85973 Kidney Cancer (OD04450-01)	1.38	1.42
Kidney	2.37	0	85974 Kidney NAT(OD04450-03)	5.71	4.64
Kidney (fetal)	0	3.93	Kidney Cancer Clontech 8120607	0.2	0
Renal ca.786-0	0	0	Kidney NAT Clontech 8120608	1.35	0.55
Renal ca. A498	0	1.91	Kidney Cancer Clontech 8120613	0.12	0.1

Renal ca. RXF 393	0	0	Kidney NAT Clontech 8120614	0.61	0.04
Renal ca. ACHN	0	0	Kidney Cancer Clontech 9010320	3.96	2.47
Renal ca. UO-31	0	0	Kidney NAT Clontech 9010321	0.45	1.12
Renal ca. TK-10	0	0	Normal Uterus GENPAK 061018	0.41	0.32
Liver	1.54	3.93	Uterus Cancer GENPAK 064011	1.23	0.94
Liver (fetal)	0	2.47	Normal Thyroid Clontech A+ 6570-1	0.58	0.81
Liver ca. (hepatoblast) HepG2	0	0	Thyroid Cancer GENPAK 064010	7.28	5.71
Lung	18.82	16.15	Thyroid Cancer INVITROGEN A302152	7.08	8.9
Lung (fetal)	3.72	3.69	Thyroid NAT INVITROGEN A302153	1.3	1.07
Lung ca. (small cell) LX-1	0	0	Normal Breast GENPAK 061019	1.14	1.83
Lung ca. (small cell)NCI-H69	0	0	84877 Breast Cancer (OD04566)	0.19	1.33
Lung ca. (s.cell var.) SHP-77	0	0	85975 Breast Cancer (OD04590-01)	2.24	2.03
Lung ca. (large cell)NCI-H460	0	0	85976 Breast Cancer Mets (OD04590-03)	6.75	8.48
Lung ca. (non-sm. cell) A549	0	0	87070 Breast Cancer Metastasis (OD04655-05)	2.66	2.66
Lung ca. (non-s.cell) NCI-H23	0	1.81	GENPAK Breast Cancer 064006	0.64	0.55
Lung ca (non-s.cell) HOP-62	0	0	Breast Cancer Clontech 9100266	0.13	0.26
Lung ca. (non-s.cl) NCI-H522	0	0	Breast NAT Clontech 9100265	0.22	0.54
Lung ca. (squam.) SW 900	0	0	Breast Cancer INVITROGEN A209073	2.29	1.73
Lung ca. (squam.) NCI-H596	2.3	0	Breast NAT INVITROGEN A2090734	0.36	0.28
Mammary gland	5.18	9.02	Normal Liver GENPAK 061009	0.63	0.22
Breast ca.* (pl. effusion) MCF-7	0	0	Liver Cancer GENPAK 064003	0.78	0
Breast ca.* (pl.ef) MDA-MB-231	0	0	Liver Cancer Research Genetics RNA 1025	0.22	0.15
Breast ca.* (pl. effusion)T47D	0	0	Liver Cancer Research Genetics RNA 1026	0.85	0.63
Breast ca. BT-549	0	0	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.36	0.52
Breast ca. MDA-N	0	0	Paired Liver Tissue Research Genetics RNA 6004-N	0.87	0.34
Ovary	16.15	9.09	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.23	0.23
Ovarian ca. OVCAR-3	0	0	Paired Liver Tissue Research Genetics RNA 6005-N	0.33	0.28
Ovarian ca. OVCAR-4	0	0	Normal Bladder GENPAK 061001	0.94	1.5
Ovarian ca. OVCAR-5	0	0	Bladder Cancer Research Genetics RNA 1023	0.11	0.38
Ovarian ca. OVCAR-8	0	0	Bladder Cancer INVITROGEN A302173	0.54	1.22
Ovarian ca. IGROV-1	0	0	87071 Bladder Cancer (OD04718-01)	1.82	1.15
Ovarian ca.* (ascites) SK-OV-3	0	0	87072 Bladder Normal Adjacent (OD04718-03)	2.18	1.94
Uterus	6.47	4.3	Normal Ovary Res. Gen.	0.62	0.19
Plancenta	48.97	32.53	Ovarian Cancer GENPAK 064008	2.92	1.67
Prostate	14.97	2.19	87492 Ovary Cancer (OD04768-07)	14.36	10.81
Prostate ca.* (bone met)PC-3	0	0	87493 Ovary NAT (OD04768-08)	1.37	0.96
Testis	2.37	11.03	Normal Stomach GENPAK 061017	0.59	1.5
Melanoma Hs688(A).T	0	0	NAT Stomach Clontech 9060359	0.65	0.56

Melanoma* (met) Hs688(B).T	0	0	Gastric Cancer Clontech 9060395	1.06	1.22
Melanoma UACC-62	0	0	NAT Stomach Clontech 9060394	1.15	0.44
Melanoma M14	0	0	Gastric Cancer Clontech 9060397	0.94	0.92
Melanoma LOX IMVI	0	0	NAT Stomach Clontech 9060396	1.71	1.14
Melanoma* (met)SK-MEL-5	3.98	4.27	Gastric Cancer GENPAK 064005	3	2.57
Adipose	15.5	16.72			

The quantitative expression of GPCR1 was also assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR; TaqMan®). RTQ PCR was performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System. In this Example, samples are referred to as Panel 4 and contain cells and cell lines from normal cells and cells related to inflammatory conditions.

Panel 4 includes samples on a 96 well plate (2 control wells, 94 test samples) composed of RNA (Panel 4r) or cDNA (Panel 4d) isolated from various human cell lines or tissues related to inflammatory conditions. Total RNA from control normal tissues such as colon and lung (Stratagene ,La Jolla, CA) and thymus and kidney (Clontech) were employed. Total RNA from liver tissue from cirrhosis patients and kidney from lupus patients was obtained from BioChain (Biochain Institute, Inc., Hayward, CA). Intestinal tissue for RNA preparation from patients diagnosed as having Crohn's disease and ulcerative colitis was obtained from the National Disease Research Interchange (NDRI) (Philadelphia, PA).

Astrocytes, lung fibroblasts, dermal fibroblasts, coronary artery smooth muscle cells, small airway epithelium, bronchial epithelium, microvascular dermal endothelial cells, microvascular lung endothelial cells, human pulmonary aortic endothelial cells, human umbilical vein endothelial cells were all purchased from Clonetics (Walkersville, MD) and grown in the media supplied for these cell types by Clonetics. These primary cell types were activated with various cytokines or combinations of cytokines for 6 and/or 12-14 hours, as indicated. The following cytokines were used; IL-1 beta at approximately 1-5 ng/ml, TNF alpha at approximately 5-10 ng/ml, IFN gamma at approximately 20-50 ng/ml, IL-4 at approximately 5-10 ng/ml, IL-9 at approximately 5-10 ng/ml, IL-13 at approximately 5-10 ng/ml. Endothelial cells were sometimes starved for various times by culture in the basal media from Clonetics with 0.1% serum.

Mononuclear cells were freshly prepared from normal human blood using standard methods known in the art. Monocytes were isolated and differentiated by methods well known in the art. CD4 lymphocytes, CD8 lymphocytes and NK cells were also isolated from mononuclear cells using CD4, CD8 and CD56 Miltenyi beads, positive VS selection columns

and a Vario Magnet according to the manufacturer's instructions. CD45RA and CD45RO CD4 lymphocytes were isolated by depleting mononuclear cells of CD8, CD56, CD14 and CD19 cells using CD8, CD56, CD14 and CD19 Miltenyi beads and +ve selection. Then CD45RO beads were used to isolate the CD45RO CD4 lymphocytes with the remaining cells being CD45RA CD4 lymphocytes. To obtain B cells, tonsils were procured from NDRI and dissected to isolate B cells which were activated using PWM at 5 μg/ml or anti-CD40 (Pharmingen) at approximately 10 μg/ml and IL-4 at 5-10 ng/ml. Primary and secondary Th1/Th2 and Tr1 cells were cultured using a standard method well known in the art. Activated Th1 and Th2 lymphocytes were maintained in this way for a maximum of three cycles. RNA was prepared from primary and secondary Th1, Th2 and Tr1 after 6 and 24 hours following the second and third activations with plate bound anti-CD3 and anti-CD28 mAbs and 4 days into the second and third expansion cultures in Interleukin 2.

The following leukocyte cells lines were obtained from the ATCC: Ramos, EOL-1, KU-812. EOL cells were further differentiated by culture: keratinocyte line CCD106 and an airway epithelial tumor line NCI-H292 were also obtained from the ATCC. CCD1106 cells were activated for 6 and 14 hours with approximately 5 ng/ml TNF alpha and 1 ng/ml IL-1 beta, while NCI-H292 cells were activated for 6 and 14 hours with the following cytokines: 5 ng/ml IL-4, 5 ng/ml IL-9, 5 ng/ml IL-13 and 25 ng/ml IFN gamma.

The primer-probe set used for expression analysis of clone GPCR1 is shown in Table 9.

The results of two replicate runs assessing the expression of GPCR1 on Panel 3 are shown in Table 11. GPCR1 is expressed in normal tissues, such as kidney, thymus, lung and colon. It is most highly expressed on resting monocytes. Surprising results relating to inflammation indicate that the expression of GPCR1 is reduced greater than 100 fold (virtually eliminated) on immune-activated monocytes.

Table 11. TaqMan results for clone GPCR1 on Panel 3.

Tissue_Name	el. xpr.	Rel. Expr. %	Tissue_Name	Rel. Expr. %	Rel. Expr. %
93768_Secondary Th1_anti-CD28/anti-CD3	1.0	1.6	93100_HUVEC (Endothelial)_IL-1b	0.0	0.0
93769_Secondary Th2_anti-CD28/anti-CD3	21.0	29.5	93779_HUVEC (Endothelial)_IFN gamma	0.0	0.0
93770_Secondary Tr1_anti-CD28/anti-CD3	5.0	10.8	93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	0.0	0.0
93573_Secondary Th1_resting day 4-6 in IL-2	0.7	3.8	93101_HUVEC (Endothelial)_TNF alpha + IL4	0.0	0.0

93572_Secondary Th2_resting day 4-6 in IL-2	13.9	15.4	93781_HUVEC (Endothelial)_IL-11	0.0	0.0
93571_Secondary Tr1_resting day 4-6 in IL-2	6.1	5.0	93583_Lung Microvascular Endothelial Cells_none	0.0	0.0
93568_primary Th1_anti-CD28/anti-CD3	0.0	2.6	93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93569_primary Th2_anti-CD28/anti-CD3	39.0	54.3	92662_Microvascular Dermal endothelium_none	0.0	0.0
93570_primary Tr1_anti-CD28/anti-CD3	10.7	21.6	92663_Microsvasular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93565_primary Th1_resting dy 4-6 in IL-2	21.0	24.7	93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.0	0.0
93566_primary Th2_resting dy 4-6 in IL-2	26.1	21.2	93347_Small Airway Epithelium_none	0.0	0.0
93567_primary Tr1_resting dy 4-6 in IL-2	11.7	19.1	93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	9.6	7.1	92668_Coronery Artery SMC_resting	0.0	0.0
93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	15.6	17.3	92669_Coronery Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93251_CD8 Lymphocytes_anti-CD28/anti-CD3	11.7	9.3	93107_astrocytes_resting	0.0	0.5
93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	8.4	9.0	93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	22.2	18.3	92666_KU-812 (Basophil)_resting	0.5	2.4
93354_CD4_none	18.6	9.1	92667_KU-812 (Basophil)_PMA/ionoycin	2.9	1.7
93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	3.8	7.6	93579_CCD1106 (Keratinocytes)_none	0.0	0.7
93103_LAK cells_resting	43.2	32.5	93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	0.0	0.0
93788_LAK cells_IL-2	15.9	18.1	93791_Liver Cirrhosis	4.8	6.3
93787_LAK cells_IL-2+IL-12	32.3	30.8	93792_Lupus Kidney	0.6	0.0
93789_LAK cells_IL-2+IFN gamma	62.9	57.8	93577_NCI-H292	0.0	0.0
93790_LAK cells_IL-2+ IL-18	39.2	52.1	93358_NCI-H292_IL-4	0.0	0.0
93104_LAK cells_PMA/ionomycin and IL-18	43.5	52.9	93360_NCI-H292_IL-9	0.0	0.0
93578_NK Cells IL-2_resting	37.4	35.6	93359_NCI-H292_IL-13	0.0	0.0
93109_Mixed Lymphocyte Reaction_Two Way MLR	26.1	23.0	93357_NCI-H292_IFN gamma	0.0	0.0
93110_Mixed Lymphocyte Reaction_Two Way MLR	17.0	10.7	93777_HPAEC_-	0.0	0.5
93111_Mixed Lymphocyte Reaction_Two Way MLR	2.2	7.4	93778_HPAEC_IL-1 beta/TNA alpha	0.0	0.0
93112_Mononuclear Cells (PBMCs)_resting	19.9	28.3	93254_Normal Human Lung Fibroblast_none	0.0	0.0
93113_Mononuclear Cells (PBMCs)_PWM	28.3	31.4	93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0	0.0
93114_Mononuclear Cells (PBMCs)_PHA-L	10.9	14.4	93257_Normal Human Lung Fibroblast_IL-4	0.0	0.0
93249_Ramos (B cell)_none	0.0	0.1	93256_Normal Human Lung Fibroblast_IL-9	0.0	0.0
93250_Ramos (B cell)_ionomycin	0.0	0.0	93255_Normal Human Lung Fibroblast_IL-13	0.0	0.0

93349_B lymphocytes_PWM	9.0	9.5	93258_Normal Human Lung Fibroblast_IFN gamma	0.0	0.0
93350_B lymphoytes_CD40L and IL-4	9.5	11.1	93106_Dermal Fibroblasts CCD1070_resting	0.0	0.0
92665_EOL-1 (Eosinophil)_dbcAMP differentiated	1.1	0.0	93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	17.1	20.3
93248_EOL-1 (Eosinophil)_dbcAMP / PMAionomycin	3.7	0.6	93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	0.0	0.0
93356_Dendritic Cells_none	3.7	10.3	93772_dermal fibroblast_IFN gamma	0.0	0.0
93355_Dendritic Cells_LPS 100 ng/ml	6.1	8.1	93771_dermal fibroblast_IL-4	1.1	0.0
93775_Dendritic Cells_anti-CD40	4.9	3.6	93259_IBD Colitis 1**	0.6	3.3
93774_Monocytes_resting	100.0	100.0	93260_IBD Colitis 2	1.1	0.0
93776_Monocytes_LPS 50 ng/ml	0.0	0.3	93261_IBD Crohns	2.2	3.5
93581_Macrophages_resting	0.6	3.7	735010_Colon_normal	21.5	15.6
93582_Macrophages_LPS 100 ng/ml	1.0	3.1	735019_Lung_none	17.6	14.4
93098_HUVEC (Endothelial)_none	0.0	0.0	64028-1_Thymus_none	11.8	7.1
93099_HUVEC (Endothelial)_starved	0.0	0.0	64030-1_Kidney_none	33.7	20.3

EQUIVALENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims which follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22;

(b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;

(c) an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22; and

(d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence.

2 The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22.

3. The polypeptide of claim 2, wherein said allelic variant comprises an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21.

4. The polypeptide of claim 1, wherein the amino acid sequence of said variant comprises a conservative amino acid substitution.

5. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22;

(b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;

(c) an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22;

(d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence;

(e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence chosen from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence; and

(f) a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e).

6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally-occurring allelic nucleic acid variant.

7. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule encodes a polypeptide comprising the amino acid sequence of a naturally-occurring polypeptide variant.

8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21.

9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21;

(b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, provided that no more than 20% of the nucleotides differ from said nucleotide sequence;

(c) a nucleic acid fragment of (a); and

(d) a nucleic acid fragment of (b).

10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to a nucleotide sequence chosen from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, or a complement of said nucleotide sequence.

11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

(a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 20% of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence;

(b) an isolated second polynucleotide that is a complement of the first polynucleotide; and

(c) a nucleic acid fragment of (a) or (b).

12. A vector comprising the nucleic acid molecule of claim 11.

13. The vector of claim 12, further comprising a promoter operably-linked to said nucleic acid molecule.

14. A cell comprising the vector of claim 12.

15. An antibody that binds immunospecifically to the polypeptide of claim 1.

16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.

17. The antibody of claim 15, wherein the antibody is a humanized antibody.

18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:

(a) providing the sample;

(b) contacting the sample with an antibody that binds immunospecifically to the polypeptide; and

(c) determining the presence or amount of antibody bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:

(a) providing the sample;

(b) contacting the sample with a probe that binds to said nucleic acid molecule; and

(c) determining the presence or amount of the probe bound to said nucleic acid molecule, thereby determining the presence or amount of the nucleic acid molecule in said sample.

20. The method of claim 19 wherein presence or amount of the nucleic acid molecule is used as a marker for cell or tissue type.

21. The method of claim 20 wherein the cell or tissue type is cancerous.

22. A method of identifying an agent that binds to a polypeptide of claim 1, the method comprising:

(a) contacting said polypeptide with said agent; and

(b) determining whether said agent binds to said polypeptide.

23. The method of claim 22 wherein the agent is a cellular receptor or a downstream effector.

24. A method for identifying an agent that modulates the expression or activity of the polypeptide of claim 1, the method comprising:

(a) providing a cell expressing said polypeptide;

(b) contacting the cell with said agent, and

(c) determining whether the agent modulates expression or activity of said polypeptide,

whereby an alteration in expression or activity of said peptide indicates said agent modulates expression or activity of said polypeptide.

25. A method for modulating the activity of the polypeptide of claim 1, the method comprising contacting a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

26. A method of treating or preventing a GPCRX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the polypeptide of claim 1 in an amount sufficient to treat or prevent said GPCRX-associated disorder in said subject.

27. The method of claim 26 wherein the disorder is selected from the group consisting of cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Ostoeodystrophy, and other diseases, disorders and conditions of the like.

28. The method of claim 26 wherein the disorder is related to cell signal processing and metabolic pathway modulation.

29. The method of claim 26, wherein said subject is a human.

30. A method of treating or preventing a GPCRX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the nucleic acid of claim 5 in an amount sufficient to treat or prevent said GPCRX-associated disorder in said subject.

31. The method of claim 30 wherein the disorder is selected from the group consisting of cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Ostoeodystrophy, and other diseases, disorders and conditions of the like.

32. The method of claim 30 wherein the disorder is related to cell signal processing and metabolic pathway modulation.

33. The method of claim 30, wherein said subject is a human.

34. A method of treating or preventing a GPCRX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the antibody of claim 15 in an amount sufficient to treat or prevent said GPCRX-associated disorder in said subject

35. The method of claim 34 wherein the disorder is selected from the group consisting of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

36. The method of claim 34 wherein the disorder is related to cell signal processing and metabolic pathway modulation.

37. The method of claim 34, wherein the subject is a human.

38. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically-acceptable carrier.

39. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically-acceptable carrier.

40. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically-acceptable carrier.

41. A kit comprising in one or more containers, the pharmaceutical composition of claim 38.

42. A kit comprising in one or more containers, the pharmaceutical composition of claim 39.

43. A kit comprising in one or more containers, the pharmaceutical composition of claim 40.

44. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:

(a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and

(b) comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease;

wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to said disease.

45. The method of claim 44 wherein the predisposition is to cancers.

46. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:

(a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and

(b) comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease;

wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.

47. The method of claim 46 wherein the predisposition is to cancers.

48. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising an amino acid sequence of at least one of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22, or a biologically active fragment thereof.

49. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.

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